

## **Historic, Archive Document**

Do not assume content reflects current scientific knowledge, policies, or practices.



urne  
47.9  
98

\*\*\*

# POULTRY RESEARCH PROGRESS REPORT

U. S. DEPT. OF AGRICULTURE  
NATIONAL AGRICULTURAL LIBRARY

Part I-c of

AUG 18 1967

Animal-Poultry and Products Research

CURRENT SERIAL RECORDS

A summary of current program and preliminary report of progress of the United States Department of Agriculture and related work of the State Agricultural Experiment Stations.

This progress report is primarily a research tool for use of scientists and administrators in program coordination, development, and evaluation; and for use of advisory committees in program review and development of recommendations for future research programs.

The summaries of research progress include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members, and others having a special interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of U.S.D.A. and cooperative research issued during the past year. Current agricultural research findings are also published in the monthly U.S.D.A. publications, Agricultural Research and The Farm Index.

UNITED STATES DEPARTMENT OF AGRICULTURE  
Washington, D. C. 20250

December 31, 1965





# Part I-c, POULTRY RESEARCH PROGRESS REPORT

## TABLE OF CONTENTS

	<u>Page</u>
Introduction. . . . .	iv
I. FARM RESEARCH	
Poultry - Breeding. . . . .	1
Poultry - Physiology. . . . .	9
Poultry - Nutrition . . . . .	18
Poultry - Improvement of Viability. . . . .	26
Poultry - Broiler Losses. . . . .	35
Poultry Performance and Management Records. . . . .	39
Production Influences on Poultry Products . . . . .	43
Infectious and Non-infectious Diseases of Poultry .	45
Parasites and Parasitic Diseases of Poultry . . . .	64
Poultry Insects . . . . .	67
II. UTILIZATION RESEARCH	
Poultry - Processing and Products . . . . .	84
Eggs - Processing and Products. . . . .	92
III. MARKETING AND ECONOMIC RESEARCH	
Poultry Products - Market Quality . . . . .	100
Poultry and Eggs - Marketing Facilities, Equipment and Methods . . . . .	105

## TABLE OF CONTENTS

	<u>Page</u>
<b>I. FARM RESEARCH</b>	
Animal Biology. . . . .	1
Fur Animal Husbandry. . . . .	20
Diseases and Parasites of Horses. . . . .	27
Infectious and Non-infectious Diseases of Fur Animals including Rabbits . . . . .	30
Miscellaneous Infectious and Non-infectious Diseases of Animals . . . . .	34
Treatment for Removal of Parasites of Domestic Animals. . . . .	50
Miscellaneous Parasites and Parasitic Diseases. .	57
Beef Cattle, Horse, and Swine Insects . . . . .	63
Livestock Engineering . . . . .	99
<b>II. NUTRITION, CONSUMER USE, AND UTILIZATION RESEARCH</b>	
Nutrition and Consumer Use Research . . . . .	123
Meats - Processing and Products . . . . .	136
Animal Fats and Oils - Industrial Utilization . .	146
Hides, Skins and Leather - Processing and Products	157
<b>III. MARKETING AND ECONOMIC RESEARCH</b>	
Livestock and Meat - Market Quality . . . . .	167
Livestock, Meat, and Wool - Marketing Facilities, Equipment and Methods . . . . .	172
Economics of Marketing . . . . .	177
Cooperative Marketing . . . . .	194
Economic and Statistical Analysis . . . . .	201
Consumer Preference and Quality Discrimination - Household and Industrial. . . . .	210

The research program pertaining to poultry is reported in two volumes: Part I-c and Part II of Animal-Poultry and Products Research. This volume contains a report of farm research applicable to the production of poultry and eggs. The companion volume, Part II, contains not only information applicable to poultry and poultry products but to other classes of live-stock, as well.

The following subject matter progress reports are prepared by U.S.D.A. The number prefixes refer to advisory committees listed later that review the research reported.

- 6 - Forestry (other than Forest Service)
- 7 - Beef Cattle, Part I-a
- 7 - Dairy, Part I-b
- 7 - Poultry, Part I-c
- 7 - Sheep and Wool, Part I-d
- 7 - Swine, Part I-e
- 7 - Animal-Poultry and Products, Part II
- 8 - Cotton and Cottonseed
- 9 - Grain and Forage Crops
- 10 - Horticultural Crops
- 11 - Oilseed and Peanut
- 11 - Sugar
- 13 - Tobacco

The information contained in the above subject matter reports was first reported in the following organizational unit reports. As above, the number prefixes refer to advisory committees listed later that review all of the work of the respective divisions or services.

Agricultural Research Service (ARS)

- 1 - Agricultural Engineering
- 1 - Soil and Water Conservation
- 2 - Utilization -- Eastern
- 2 - Utilization -- Northern
- 2 - Utilization -- Southern
- 2 - Utilization -- Western
- 3 - Human Nutrition
- 3 - Clothing and Housing
- 3 - Consumer and Food Economics
- 4 - Market Quality
- 4 - Transportation and Facilities
- 7 - Animal Husbandry
- 7 - Animal Disease and Parasite
- 12 - Crops
- 12 - Entomology

Economic Research Service (ERS)

- 1, 5 - Economic Development
- 4, 5 - Marketing Economics
- 5 - Farm Production Economics
- 5 - Economic and Statistical Analysis
- 5 - Foreign Development and Trade
- 5 - Foreign Regional Analysis
- 5 - Natural Resource Economics
- 6 - Forest Service - Research (FS)
- 4, 5 - Farmer Cooperative Service (FCS)
- 4, 5 - Statistical Reporting Service (SRS)



The research program of the Department of Agriculture is reviewed annually by the following advisory committees:

1. Farm Resources and Facilities Research
2. Utilization Research and Development
3. Human Nutrition and Consumer Use Research
4. Marketing Research
5. Agricultural Economics Research
6. Forestry Research
7. Animal and Animal Products Research
8. Cotton Research
9. Grain and Forage Crops Research
10. Horticultural Crops Research
11. Oilseed, Peanut and Sugar Crops Research
12. Plant Science and Entomology Research
13. Tobacco Research

A copy of any of the reports may be requested from Max Hinds, Executive Secretary, Animal and Animal Products Research Advisory Committee, Research Program Development and Evaluation Staff, U. S. Department of Agriculture, Washington, D. C. 20250

## INTRODUCTION

The poultry research program reported in Part I-c and Part II, Animal-Poultry and Products Research Progress Reports, covers work directly related to the production, processing, distribution, and consumption of poultry and poultry products. The information has been assembled from the organizational unit reports of the several divisions. This report does not include extensive cross commodity work, much of which is basic in character, which contributes to the solution of not only poultry problems but also to the problems of other commodities. Progress on cross commodity work is found in the organizational unit reports of the several divisions.

These reports are organized by "Problem Areas" which are shown in the table of contents. For each area there is a statement of (1) the problem, (2) the USDA program, (3) State experiment station programs, (4) a summary of progress during the past year on USDA and cooperative work, and (5) a list of publications resulting from USDA and cooperative work.

Research on Animal-poultry and products problems is supported by (1) Federal funds appropriated to the research agencies of the USDA, (2) Federal and State funds appropriated to the State agricultural experiment stations, and (3) private funds for research carried on in private laboratories or for support of State station and USDA work.

### Research by USDA

Farm research pertaining to poultry is conducted in the Agricultural Research Service Divisions of Agricultural Engineering, Animal Disease and Parasite, Animal Husbandry, and Entomology. The work comprises investigations of breeding, physiology, nutrition, viability, diseases, insects, housing and management, involving an estimated 75 professional man-years of scientific effort.

Nutrition, consumer, and utilization research pertaining to poultry is conducted in the Agricultural Research Service divisions of Human Nutrition, Consumer and Food Economics, and Western Utilization. The work comprises investigations of composition and nutritive value; physiological availability of nutrients and their effects; new and improved methods of preparation, preservation, and care in homes, eating establishments and institutions; and with the processing phase involving slaughtering the birds and processing the meat and eggs. Also, it is concerned with improved equipment and processes. The work in these divisions involves an estimated 32 professional man-years of scientific effort.

Marketing and economic research pertaining to poultry is carried on within four Services: Agricultural Research Service, Economic Research Service, Farmer Cooperative Service, and Statistical Reporting Service. The work comprises (1) physical and biological aspects of assembly, packaging, transporting, storing and distribution; (2) economic aspects of marketing



costs, margins and efficiency, market potential, supply and demand, and situation and outlook; (3) cooperative marketing, and (4) consumer acceptance studies. The divisions in which the work is conducted are: Market Quality, ARS; Transportation and Facilities, ARS; Marketing Economics, ERS; Economic and Statistical Analysis, ERS; Marketing Division, FCS; Standards and Research, SRS. The scientific effort involved by these divisions applicable to poultry is estimated at 26 professional man-years.

### Interrelationships among Department, State and Private Research

A large part of the Department's research is cooperative with State Experiment Stations. Many Department employees are located at State Stations and use laboratory and office space close to or furnished by the Station. Cooperative work is jointly planned, frequently with the participation of representatives of the producers or industry affected. The nature of cooperation varies with each study. It is developed so as to fully utilize the personnel and other resources of the cooperators which frequently includes resources contributed by the interested producers or industry.

Including both cooperative and State Station projects poultry research is carried on by 51 State Experiment Stations. The types of work to which the largest amount of effort is devoted include nutrition, physiology and management, breeding, diseases and economics of marketing. There is regular exchange of information between Station and Department scientists to assure that the programs complement each other and to eliminate unnecessary duplication.

Privately supported poultry research emphasizes the solution of scientific production, processing, and marketing problems. Much of it utilizes the results of more basic work done by State Station and Department scientists.

Major areas of emphasis include poultry nutrition and breeding. This is peculiar to the poultry industry. For other animal species much of the nutrition and breeding work is done with public research. Private research in processing is devoted largely to control methods, standardization of products, and product quality and formulation. Research in marketing and economics by industry is in connection with new product development and in merchandising and promoting farm products. Industry participates heavily in consumer acceptance research but largely with respect to a firm's own brand name.

The contributions of poultry producers and industry to the work of the State Stations and the Department have been an important factor in the success of their research programs. Producers offer flocks and facilities for testing products and practices used in production. Likewise, processors and retailers offer facilities and products for use by public research agencies. Many problems in the economics of marketing cannot be transferred to a laboratory, experimental plot, or other simulated situa-



tion. The results of economic research conducted cooperatively is of great value to industry, especially in cases where public research can provide comparison and analysis. Even large firms that have a research staff do not have access to the plants and records of competitors.

### Examples of Recent Research Accomplishments by USDA and Cooperating Scientists

Parthenogenesis increased in presence of live virus. A possible relationship between certain live viruses and parthenogenesis in turkeys and chickens has been observed at Beltsville. Fowl pox, Rous sarcoma, and Newcastle disease viruses, each has been shown to be effective in enhancing parthenogenetic development in turkeys. When the viruses were inactivated with beta propolactone prior to inoculation into turkeys, they proved ineffective in enhancing parthenogenesis. Spontaneous parthenogenesis in unfertilized White Leghorn eggs also has been noted following a natural outbreak of visceral lymphomatosis. The observation that live viruses will stimulate cell division and growth will be useful to the research worker but also may have far-reaching implications in animal and human health.

Breeding for resistance to leukosis. Relatively simple breeding procedures for developing strains of chickens resistant to the growth of leukosis viruses are suggested by research findings at the Regional Poultry Research Laboratory at East Lansing, Michigan, and at other laboratories. Two different single genes have been identified, each having a somewhat different effect on the antigenic response. Homozygous recessive chickens, or cell cultures from such chickens, have a greatly reduced ability to support growth of certain specific viruses. While these results are promising, further tests on the effects of these genes on neoplasms occurring under widely different conditions of exposure are necessary before specific recommendations can be made to poultry breeders.

Ventilation of livestock buildings. Research in cooperation with State experiment stations has obtained much needed basic data on the heat and moisture given off by cattle, hogs, and poultry, and on the influence of building environment on production and feed consumption. The heat and moisture dissipation data are considered basic design data for ventilation systems of poultry, dairy, and swine buildings. They appear in design handbooks including the 1965 Guide and Data Book of the American Society of Heating, Refrigeration, Ventilating, and Air Conditioning Engineers, and are used by makers of ventilating equipment, prefabricated buildings and package buildings as well as by specialists advising farmers on their own construction. Building improvements resulting from the above research have contributed to the substantial rise in efficiency of livestock production that has occurred during the past decade.



Immunizing antigens of *Pasteurella multocida*. Particulate antigens were isolated from two distinct immunogenic strains of *Pasteurella multocida* of avian origin. An emulsified vaccine prepared with antigens from one of the strains induced 100% immunity in chickens to an homologous challenge which killed 100% of the unvaccinated controls. The antigens possessed many of the chemical and physical properties ascribed to endotoxins. Injections of fractional milligram amounts of the antigens into chickens produced signs which are usually observed in acute cases of fowl cholera, such as depression, increased salivation, diarrhea, and some times death.

Development of a Rapid Method of Analyzing Egg Products for Salmonella Using the Fluorescent Antibody Technique. The presence of Salmonella in food products and in raw materials can now be detected quickly and easily as a result of Department research. Other currently used assay methods for detecting Salmonella in eggs and egg products are both tedious and time consuming. Complete assays require from three to five days. A fluorescent antibody technique has been applied to the development of an assay method for Salmonella in egg products that can be completed in 10 to 24 hours. In this method specific Salmonella antibodies, obtained from rabbits and guinea pigs, and labeled with fluorescent dyes, become concentrated around any Salmonella cells present and appear as fluorescent spots on microscope slides. Other microorganisms do not react with the antibody, and no fluorescent spots are evident. The new method compares favorably with existing methods both in reliability and sensitivity and represents another step in our drive to eliminate Salmonella from food products.

Safety of consumer procedures for roasting stuffed turkeys evaluated. Covering the breast of turkeys during roasting slowed the rate of heat penetration in this area and increased the total cooking time needed to insure destruction of any food poisoning organisms in the stuffing. Beltsville Small White and Bronze tom turkeys weighing 13 to 16 pounds and 16 to 23 pounds respectively were studied. Breast temperatures of 195° F. at end of cooking followed by a 20 minute holding period insured microbial safety of the stuffing. Frequently recommended cooking procedures giving endpoint temperatures of 185° F. in the breast or inner thigh were not always adequate to raise the stuffing temperature to 165° F. considered necessary for microbial safety. The final temperatures of the stuffing as well as that of the turkey should be considered to assure optimum doneness and safe eating.

Effect of Various Factors on Moisture Absorption, Retention and Loss by Fryer Chickens. Absorption, retention and loss of moisture from chicken fryers during commercial processing, transportation and storage was found to be influenced by (a) type of cuts used during evisceration, (b) temperature of prechilling, (c) time of prechilling, (d) carcass weight, (e) washing, (f) removal of neck before chilling, (g) time of vat chilling, (h) type of chilling system, (i) phosphates added to chill water, (j) NaCl added to chill water, (k) initial pH of chill water, (l) air agitation in chiller, and (m) combination of continuous chilling with subsequent phosphate soak. These findings were utilized in promulgating many new regulations which defined procedures and tolerances related to moisture added or lost during processing and marketing of poultry. They are also available to the poultry processing industry through government, technical and trade journal publications. They assist the processor in determining the effect on product moisture of modifications in techniques or equipment.





## I. FARM RESEARCH

### POULTRY - BREEDING

#### Animal Husbandry Research Division, ARS

Problem. Poultry breeders have made tremendous progress in recent years through the application of new genetic principles revealed by basic research. Improvement in many of the economic traits has attenuated and new methods of releasing useful genetic variation are needed. Information is needed as to the relative rates of progress which will result from various breeding systems for improving such economic traits as egg and meat production. Furthermore, information is needed as to whether different breeding systems are required or are more efficient at different stages of the breeding program. In order to design the most efficient breeding systems, knowledge of the heritabilities, genetic correlations between traits, and the effects of genotype-environment interactions are required. Information on the cytogenetics of the fowl is seriously lacking and is needed for continued progress. The biochemical and physiological basis for the action of certain genes is unknown and information regarding these processes would lead to a better control of heredity for optimum performance. The economics of production should be improved through knowledge gained on the genetic aspects of feed utilization and on selection under various stress conditions.

#### USDA AND COOPERATIVE PROGRAM

This is a continuing long-term program involving basic and applied studies of the inheritance of egg production and broiler characteristics. Scientists with majors in genetics, cytology or biochemistry and minors in physiology or statistics are involved. Much of the research is conducted within the framework of four regional projects. In addition to major contributions to the establishment and maintenance of central facilities, the USDA also provides coordinating personnel located at Athens, Georgia; Lafayette, Indiana; and Beltsville, Maryland. The close working relationship between the USDA and State experiment stations in the four regional projects provides for integrated research on a large scale without duplication of effort. Research at Beltsville, Maryland, involves the selection of lines under stress of nutritional deficiency and selection for differences in feed utilization efficiency. A study of genetic, biochemical and physiological differences between these lines is also in progress. Selection for response in egg production to "18-hour" days is conducted in cooperation with AERD. Research in the North Central region is primarily on egg production traits and is done at the Regional Poultry Breeding Laboratory, Lafayette, Indiana, and at 12 cooperating State experiment stations. In the Southern region the emphasis is divided between egg and broiler traits and the work is conducted at the Southern Regional Poultry Genetics Laboratory, Athens, Georgia, and at 14 cooperating State experiment stations. The work in the Northeastern region involves the improvement

of chickens through genetic and physiological studies and is conducted under cooperative projects at 11 cooperating State experiment stations. Cooperative work on reproductive problems of turkeys is carried on with three Western States.

Public Law 480 research projects are in progress or have been initiated as follows: The Animal and Poultry Breeding Department, Ministry of Agriculture, Dekki Gisa, Egyptian Region, U.A.R., is conducting a study to improve and evaluate the Fayoumi and Dandarawi fowl. A study of the effect of environmental stresses on *Tribolium* is being conducted in Spain at the Instituto Nacional de Investigaciones Agronomicas, Madrid, Spain. The Hebrew University, Faculty of Agriculture, Rehovot, Israel, is studying bacteriological problems involved in artificial insemination of hens. A study to evaluate native Indian fowl as new sources of germ plasm is being conducted by the Punjab Agricultural University, Ludhiana, India.

A total of 7.0 professional Federal man-years is devoted to this program annually. Of this number 3.9 man-years are devoted to genetics and inter-relations of performance traits, and 3.1 to selection and systems of breeding.

#### PROGRAM OF STATE EXPERIMENT STATIONS

Considerable poultry breeding research is cooperative in the regional poultry breeding projects. NC-47 is concerned with the evaluation of different breeding systems, primarily for egg production. Scientists in NE-51 are examining several physiological traits in relation to the genetics of efficient production of eggs and meat. S-57 is designed to evaluate the relative importance of genetic-environmental interactions in meat production and egg production phases as well as environmental effects on parent flocks. W-78 is concerned with the influence of altitude on the genetic capabilities of turkey reproduction. The contributors to regional projects make use of randombred populations as genetic controls, and the effectiveness of these controls is being evaluated.

Research emphasis is given to quantitative genetics and selection under stress. Fertility, hatchability and other reproductive problems are studied intensively, especially in turkeys. Some stations are concerned with the genetics of disease resistance. Breakthroughs of production ceilings are sought using cell irradiation, different lighting regimes, and new biochemical measurements. Experiments on cytogenetics of poultry promise new knowledge of the chromosome complement and identification of some of the known linkage groups with their particular chromosomes.

The total State scientific effort devoted to poultry breeding research is 50.0 professional man-years.



## PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Genetics and Interrelations of Performance Traits

1. Genetic aspects of nutritional deficiencies. Selection has been practiced for four generations for fast and slow growth rate on both a methionine deficient diet and a normal diet. Progress has been made with the divergent selection on both diets as compared to a randombred control population. The chicks reared on the deficient diet had a growth rate at 3 weeks of age of less than half that of chicks on a normal diet. Large differences were observed between the selected lines. Chicks reared to 3 weeks on a deficient diet did not compensate for the reduced growth when fed a normal diet from 3-through 8-weeks. Preliminary evidence indicates little or no difference among the lines in methionine requirement. (AH el-48)

2. Biochemical basis for genetic differences in growth rate. Lines of chickens established by selection for fast or slow growth on a methionine deficient diet do not differ in either methionine or protein requirements. In addition, little difference in growth rate is observed when chicks from each line are fed equivalent quantities of feed. These results indicate that selection progress on a methionine deficient diet is accomplished through a mechanism involving heritable differences in feed consumption rather than efficiency of nutrient utilization. Progeny from 4 dam families within each of 25 sire families were tested for differential growth performance when each dam family was provided daily with an identical quantity of methionine deficient diet. Three-week body weights indicate that the chicks from the best sire family gained an average of 71 grams while the chicks from the poorest sire family gained an average of 59 grams. A sire component analysis showed that the heritability estimated for efficiency of gain on identical quantities of methionine deficient diet was 0.66. (AH el-52)

3. Selection for egg production under sub-circadian periodicities. Egg production for the third generation of White Leghorns selected under 18-hour "short days" continued to show a small increase over previous generations. Hen-day percent egg production was 1.8 and 3.4 percentage points greater than the previous year for the 18-hour and control population, respectively. Egg production for both populations was 4.5 percentage points greater than the randombred control when measured as percent deviation from the randombred control. Differences noted in the third generation, between the 18-hour and control population continued to be of low magnitude. Birds in the 18-hour population, however, continued to be lighter in body weight at 8 weeks of age and reached sexual maturity later than the control population. (AH el-47)

4. Genotype-environment interactions. Studies to identify specific genotype-environment interactions in both egg and meat production stocks were conducted as a part of the Southern Regional Poultry Breeding Project. Recently two trials were conducted with experiment station meat stocks housed at different growing locations (States) under one-half and one square foot of floor space per bird. Data were analyzed separately by sex. Genotype (stock) by environment (locations, floor space treatments) interactions were not significant. Stock by trial interactions were significant at eight weeks of age, but made up only 2.7 and 1.0% of the total variation for males and females, respectively. Studies with egg and meat production stocks are currently being analyzed to estimate the effect of parent flock source and location on the magnitude and relative importance of genotype-environment interactions. (AH el-44)

The North Carolina station tested different genotypes involving both broilers and layers in each of three years at each of three locations. Results with layers indicated highly significant location by year and genotype by year interactions were present for egg production, body weight at sexual maturity, and fall and spring egg weights. Genotype by location interactions were significant for fall egg weight, while the second order interactions were significant for all characters except body weight. The importance of these interactions is reflected in the effect of different testing procedures on genetic progress. (AH el-44)

## B. Selection and Systems of Breeding

### 1. Evaluation of genetic changes produced by various breeding systems.

Studies designed to evaluate breeding systems and selection methods were continued at the central facilities of the North Central Regional Poultry Breeding Laboratory and at five cooperating State stations. Three random-bred control populations are maintained and made available to cooperators as foundation stock and controls. Eggs from these control stocks are shipped to many locations throughout the country upon request. Selection at each station is for one trait, hen-day percent egg production to about 300 days of age. Other traits are observed but are not the basis for selection. Selection pressure in the project is maintained at approximately 25%.

The Indiana station has completed three generations of closed flock index selection (based on sire, dam, and family means) and reciprocal recurrent selection involving the Purdue Pool and the Regional Cornell populations. Third generation tests have also been made of crosses of the index selected lines and of index selection within a cross of the two control populations. These tests indicate little or no progress in hen-day percent egg production from reciprocal recurrent selection or from index selection within the cross. Index selection within the two parent populations and crossing



of the two selected lines seem to show some improvement in egg production. However, it appears that egg size in all the selected lines has decreased. A similar project is in progress at the Kansas Station, except that the Regional Red is used as one of the parent lines rather than the Purdue Pool. Results indicate that family index selection has been more effective than reciprocal recurrent selection in increasing egg production. This difference appears to be relatively consistent for both the selected strains and for their crosses. Data for hen-day percent egg production to 500 days of age is similar to that for the selected trait, hen-day percent production to 260 days of age. Egg size and body weight seem to have decreased in the populations showing increased egg production.

The Missouri station continued the following selection systems through the fifth generation: (1) intraflock in which selection is based on an overall performance index; (2) recurrent selection to three inbred male tester lines (L1, R3, W4); and (3) selection within three Leghorn female lines (La, Lc, Ld). Randombred controls were produced from eggs shipped from the central facilities. Based on survivors production from 22 to 64 weeks, intraflock, crosses from the recurrent selection lines, and within line selection exceeded the control line by 10, 7 and 3 percentage points, respectively. The R3 x Lc cross exceeded the L1 x La and W4 x Ld crosses by 6 percentage points; however, this advantage was evident the first generation and may be due only to superior heterosis. Among the three female lines, the La population was about .5 pounds heavier than the other two, although all three lines were derived from the same base population five generations back and no direct selection has been practiced for body size.

Inbreeding with and without selection was continued at South Dakota and Wisconsin. Selection at Wisconsin has not been effective in offsetting the decline in egg production due to inbreeding. In three of four South Dakota comparisons, selected inbred lines outperformed unselected lines. Other major effects of inbreeding have been delayed maturity, floor laying, reduced body size and high mortality.

Forty-three populations were compared under a common environment at the Regional Laboratory. A sample of 90 birds in three replications of 30 each was tested when available. Sixteen economic traits were measured. The three major control populations, Regional Cornell (CC), Regional Red (RR) and Purdue Pool (PP), continued to remain relatively stable for all traits measured. All of the 15 selection systems based on the CC population ranked above their control in the trait of major interest, hen-day percent egg production to 500 days of age. However, only the individual selection system was significantly ( $P < .05$ ) above the control. It seems that individual selection may be more effective than family selection and that selection for combinability by recurrent or reciprocal recurrent methods may not be effective. However, more data are required for confirmation.

Some possible trends were apparent among correlated responses as the number of selected generations increased. The Iowa High Body Weight line was significantly later in age at sexual maturity ( $P \leq .05$ ) from all other lines. The sire family selection line tended to be later in age at sexual maturity than the control, whereas the dam family selection line appeared to be earlier. Egg and body weights continue to display some depression as a result of selection for early egg production. Albumen quality seemed to be lowered by selection. Of interest here is the low ranking in albumen quality of the low egg weight and low body weight lines as compared to the high egg production, high egg weight and high body weight lines. A large differential in laying house mortality existed between reciprocal crosses of CC x RR and CC x PP. Mortality was higher whenever CC males were used; 20 percentage points in CC x RR crosses and 8 percentage points in CC x PP crosses.

At the Minnesota Station, the Regional Cornell has been maintained for eight generations in two flocks, one consisting of 50 males and 250 females and the other of 15 males and 125 females. Egg production in the latter flock was somewhat less, which may be due to higher inbreeding. Also, sexual maturity was delayed in the latter flock, with little or no differences in other traits measured. (AH el-43)

2. Randombred control populations. Five randombred control populations were maintained for use by cooperators as a gene pool and as genetic and environmental controls. Three populations are maintained at Lafayette, Indiana, primarily for egg production research and two at Athens, Georgia, for meat production research. Hatching eggs from these stocks are supplied to cooperating universities, to random sample tests, to universities outside the region, and to commercial poultry breeders. (AH el-43, 44)



## PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Genetics and Interrelations of Performance Traits

- Eisen, E. J. 1964. A study of genetic combining ability among inbred lines of poultry in single crosses, double crosses and top crosses. Ph.D. thesis. Purdue University Library. (AH el-43)
- Lepore, P. D., Siegel, P. B. and Siegel, H. S. 1963. The influence of selection for growth upon the DNA and RNA concentration of chicks and chick tissues. Poultry Sci. 42:1284 (Abs.). (AH el-44)
- Lepore, P. D., Siegel, P. B. and Siegel, H. S. 1965. Nucleic acid composition of chicks and chick tissues from growth selected lines of White Rocks. Poultry Sci. 44:126-130. (AH el-52)
- Lepore, P. D. and Marks, H. L. 1965. Genetic variation of some chemical components of Coturnix quail egg yolk. Poultry Sci. 44:184-186. (AH el-52)
- Lepore, P. D. 1965. Methionine and protein requirements of lines of chickens established by growth-rate selection on a methionine deficient diet. Poultry Sci. 44:797-803. (AH el-52)
- Lowe, P. C. and Wilson, S. P. 1964. Mortality of inbred and non-inbred chickens on range. British Poultry Sci. 6:89-91. (AH el-43)
- Lowe, P. C., Wilson, S. P. and Harrington, R. B. 1964. Association of some qualitative and quantitative traits in chickens. Poultry Sci. 44:106-112. (AH el-43)
- Marks, H. L. and Kinney, T. B., Jr. 1964. Measures of egg shell quality. Poultry Sci. 43:269-271. (AH el-47)
- Marks, H. L. and Lepore, P. D. 1965. A procedure for artificial insemination of Japanese quail. Poultry Sci. 44: (Abs.). (AH el-47)
- Thomas, J. E. and Gyles, N. R. 1964. Use of the Athens-Canadian random-bred control to reduce yearly environmental fluctuations in a selection study. Poultry Sci. 43:1370-1371. (AH el-44)
- Tindell, D. 1964. Methods of reducing social stress, fighting and subsequent mortality of confinement reared cockerels. Poultry Sci. 43:1222-1225. (AH el-44)

Selection and Systems of Breeding

- Bohren, B. B. and McKean, H. E. 1964. Relaxed selection in a closed flock of White Leghorns. *Genetics* 49:279-284. (AH el-43)
- Carson, J. D. 1964. Natural selection as a genetic tool for improving hatchability in turkeys. *Poultry Sci.* 43:87-94. (AH el-46)
- Festing, M. F. 1964. The response to selection for body weight and egg weight in the fowl. Ph.D. thesis on file, Iowa State University Library. (AH el-43)
- Jaap, R. G. 1963. Selection for rapid growth rate in chickens. *Poultry Sci.* 42:1393-1397. (AH el-43)
- Marion, W. W., Nordskog, A. W., Tolman, H. S. and Forsythe, R. H. 1964. Egg composition as influenced by breeding, egg size, age and season. *Poultry Sci.* 43:255-264. (AH el-43)
- Nordskog, A. W., Festing, M. F. and Wehrli, M. 1964. Results from a 7-year selection experiment. *Poultry Sci.* 43:1347 (Abs.). (AH el-43)
- Nordskog, A. W. and Geisbrecht, F. G. 1964. Regression in egg production in the domestic fowl when selection is relaxed. *Genetics* 50:407-416. (AH el-43)
- Wehrli, M. 1964. Effects of artificial selection on reproductive fitness in the fowl. Ph.D. thesis on file, Iowa State University Library. (AH el-43)



POULTRY - PHYSIOLOGY  
Animal Husbandry Research Division, ARS

Problem. Continuing basic research in avian physiology is essential to establish fundamental concepts and to increase the knowledge upon which ultimately must depend the solution of such problems as fertility, hatchability, growth, and egg production. Basic physiological knowledge is necessary also for implementation of the subject matter of other disciplines. In reproductive physiology, for example, the dominant role of the central nervous system is now generally recognized, but much intensive research will be required before we can expect useful knowledge of mechanisms by which the varying actions of external and internal factors are integrated and directed to initiate, maintain, or modify reproductive functions. Many aspects of environmental physiology, of responses to stress, and of growth and development likewise depend upon basic research. On the more immediately practical side, increased knowledge of poultry housing, related equipment, and other management factors is necessary to provide optimal ranges of operational efficiency.

USDA AND COOPERATIVE PROGRAM

This is a continuing program, mainly on basic aspects of the physiology of avian reproduction, but including also applied studies pertaining to environmental physiology and management. In addition to physiologists, the work draws upon geneticists and animal husbandmen. Research is in progress at Beltsville, Maryland, and Glendale, Arizona, the work at Glendale contributing to regional project W-50. Cooperation currently is maintained with members of the Farm Electrification Branch, AERD; Inspection Branch, Poultry Division, C&MS; the National Institutes of Health; and Pennsylvania State University. Under PL 480, a five year project (1964-1969) on the effect of growth hormone on fat metabolism in adult hens was initiated at the Agricultural University, Warsaw, Poland. Federal research in this area calls for 6.0 professional man-years, distributed to subareas as follows: Physiology of reproduction, 4.0; and environmental physiology, 2.0.

PROGRAM OF STATE EXPERIMENT STATIONS

Poultry physiologists are studying the nervous system in order to better control physiological functions. Research on the nerve supply to the ovary and oviduct and the control of blood pressure and the circulatory system is under way. Cholesterol and fatty acid metabolism, biochemistry of egg formation, physiology of digestion and absorption, and taste are under investigation.

Research on growth and development of the embryo includes studies of how fertilization and parthenogenesis trigger embryonic development.

Scientists are probing factors controlling cell growth and the effects of pesticides on embryos.

Endocrine physiology research highlights the thyroid and adrenal glands and the bursa of Fabricius. Interrelationships of these glands with the ovary and testis and the effects of stress are important. Studies of hormonal control of egg laying continue.

Research is extended on the problems of fertility and hatchability. Efforts are continuing in hopes of perfecting a technique to permit long-term storage of poultry semen. Poor fertility is especially important in turkeys and artificial insemination is widely used. Regional project W-50 coordinates research in the western region on environmental factors affecting reproduction in chickens.

The total State scientific effort devoted to poultry physiology research is 51.5 professional man-years.

#### PROGRESS -- USDA AND COOPERATIVE PROGRAMS

##### A. Physiology of Reproduction

1. Neuroendocrinology. A recent, widely accepted theory of the neuroendocrine control of sequential ovulations in the chicken is based on the assumption that nerve impulses originating from the oviduct during passage of an egg govern the time of release of pituitary ovulation hormone (OH) for the next ovulation. Critical evaluation of this theory has awaited the development of a nonsurgical method for detecting ovulation when the ovulated egg (yolk) does not enter and traverse the oviduct. Such a method, developed at Beltsville, employed time-amplitude ultrasonography of the type used with limited success to detect soft-tissue lesions in humans. Briefly, narrow beams of high frequency sound are pulsed into the ovary from a transducer placed on the body wall. As the sound waves strike the walls of large ovarian follicles, characteristic echos are returned to the transducer, converted into electrical impulses, and displayed as tall upward spikes on an oscilloscope screen. Ovulation is detected by the abrupt disappearance of such spikes from the region of the ovulating follicle.

The ultrasonographic method was used to study the timing of sequential ovulations in hens following transection of the oviduct at the infundibulum to prevent the engulfment of eggs. Two or 3 ovulations were witnessed on consecutive days in each of several operated hens. Since all ovulations occurred within the hours predicted from past records of lay, the results argue against the existence of the postulated oviducal controls on OH release. (AH e3-21)

The hen's coupled ovulation cycle includes a single day of lapse between the terminal ovulation of one sequence and the first ovulation of a following



sequence. The uncoupled cycle is the first to appear at sexual maturity or following an interruption of two or more days. In coupled cycles, the release of gonadotrophic hormone (GTH) for ovulation of the terminal follicle of one sequence appears also to initiate maturation of the first or  $C_1$  follicle of the succeeding cycle or sequence. The maturation course of this  $C_1$  follicle and the timing of release of GTH for its ovulation might conceivably be affected by influences associated with the terminal ovulation of the preceding cycle or with the ruptured follicle or oviducal egg resulting from the terminal ovulation. In the uncoupled cycle, the release of GTH for maturation of the  $C_1$  follicle and subsequently for its ovulation both take place in absence of a preceding ovulation, ruptured follicle, or oviducal egg. Comparison of times of oviposition in 1, 2, and 3 member coupled and uncoupled cycles has afforded some evidence for the conclusion that the release of GTH for maturation of the uncoupled  $C_1$  follicle occurs during a "critical period" incident at about the hour of GTH release for maturation of the  $C_1$  follicle in lengthy coupled cycles. If so, the GTH release for ovulation of the uncoupled  $C_1$  follicle in cycles of 1, 2, and 3 members occurs on the second day following GTH release for  $C_1$  maturation, but earlier in the day by roughly 5, 8, and 9 hours respectively. The most likely means by which this advancement in the hour of GTH release for ovulation of the uncoupled  $C_1$  follicle would appear to be through the action of ovarian feedback hormones on some neural component of the GTH release mechanism.

As part of continuing research on the role of the posterior lobe of the pituitary gland on oviposition in chickens, an examination was made of the long-term morphological and functional effects of removal of the posterior lobe. The results confirmed an earlier report by other investigators that posterior lobectomy leads to persistent diabetes insipidus, but causes only temporary suspension of ovulation and lay. Further, postoperative recrudescence of the ovary as well as resumption of lay appeared to coincide with reorganization of the proximal pituitary stalk into a miniature posterior lobe, a phenomenon observed in several other vertebrate forms but not yet reported for birds.

Examination of the supraoptic and paraventricular nuclei of the hypothalamus, generally regarded as the probable sites of production of posterior lobe hormones, showed that posterior lobectomy led to a rapid depletion of cells in the supraoptic nuclei, involving a maximum of 50% of total cells within 10 days, but had only insignificant effect on cells of the paraventricular nuclei. Conversely, selective removal of the anterior pituitary gland resulted in a marked depletion of paraventricular cells without appreciable effect on cells of the supraoptic region. The apparent association of cell loss with loss of function supports other evidence indicating that in birds, as in mammals, the supraoptic nuclei produce posterior lobe hormones, while the paraventricular nuclei secrete neurohumors modulating anterior pituitary function. (AH e3-21)



2. Reproduction in Japanese quail. A detailed study of ovulation and lay in Coturnix has shown significant differences in the timing of sequential ovulations in this species and in the chicken under a 14-hour light-day. Coturnix ovulate the first ( $C_1$ ) egg of a sequence at some 8-9 hours after the onset of light, or at about 1 to 4 hours later than ovulation of the terminal ( $C_t$ ) ovulation in White Leghorn chickens. Lag, or the clockwise difference in times of day between consecutive ovulations, was considerably shorter in Coturnix than in chickens. Total lag, or the difference in times of day between  $C_1$  and  $C_t$  ovulations, ranged, in sequences of increasing length, from 1.6 to 2.9 hours in Coturnix as compared to 4.5 to 8 hours for chickens. The origins of these differences are unknown. A demonstration that the intervals from injection of OH preparations to ovulation in Coturnix and chickens are about the same would seem to rule out the possibility that the differences arise from differences in the interval from normal release of OH to ovulation. Other possibilities include differences in the rates of growth of ovarian follicles, or in times of cyclic release of pituitary hormones for maturation or ovulation of follicles. The existence of these differences suggests that Coturnix will be an especially useful subject for comparative studies on ovulation. (AH e3-22)

A method of transbuccal hypophysectomy was developed for Coturnix. The effects of hypophysectomy on the gonads, adrenals, and thyroid glands of the quail were similar to those reported for chickens. A striking difference was observed, however, in postoperative survival between the two species. Whereas mortality in chickens maintained under the most favorable environmental conditions may reach 80% within the first 2 weeks after hypophysectomy, mortality in 40 Coturnix maintained under normal laboratory conditions was less than 5% during the first 30 days. The quail resumed normal activity within 3 or 4 days and appeared hardy enough to withstand shipment to distant points. Assuming that good survival during shipment can be demonstrated, hypophysectomized Coturnix might be made commercially available and thus serve as a convenient and highly useful subject for endocrine studies in birds. (AH e3-22)

3. Parthenogenetic reproduction. A total of 6521 unfertilized Beltsville Small White turkey eggs were examined for parthenogenesis during the 1965 test period, January - May. These eggs were produced by 106 young, virgin hens representing 7 sire families. The average incidence of parthenogenesis of all eggs tested was 43.2%. The incidence of parthenogenesis in eggs of hens from the 7 different sire families varied from 39.5% to 47.1%.

A total of 708 unfertilized eggs contained well-developed embryos, of which number 223 or 31% survived for 20 days or longer within the shell. Fifty three parthenogenetic poults were hatched. These came from eggs of 31 of the 106 hens on test. One hen produced 7 poults and another 5 during the test period. Two of the 31 hens produced 3 poults each while each of 10



others were credited with 2 poults. Each of the remaining 17 hens produced only one poult.

Mortality among parthenogenetic poults continues to be high. Of the 53 poults hatched in 1965, only 9 were still alive on July 1. Perosis, the principal cause of death, appears to be largely of a hereditary nature. General muscular weaknesses, morphological abnormalities, and heart and circulatory involvement were likewise factors contributing to the heavy mortality. (AH e3-19)

Freshly laid unfertilized eggs from a number of different breeds, varieties, and strains of chickens were incubated for 8-10 days and subsequently broken and examined macroscopically for parthenogenetic development. Marked differences were found among chickens of different breeds and strains in their ability to produce eggs capable of undergoing parthenogenetic development. No parthenogenetic development was detected in eggs produced by Araucana, Old English Black Breasted Game, and New Hampshire hens. A very low incidence of parthenogenesis (less than 0.2%) was encountered in eggs from Barred Plymouth Rocks, Rhode Island Reds, and White Leghorns. The highest incidence of parthenogenesis (6.38%) was found in eggs of the Beltsville strain of Dark Cornish, eggs of the Silver Cornish ranking second with 2.26%. Eggs of two commercial strains of Dark Cornish showed 0.66% and 0.38% parthenogenesis. Of the 1143 eggs classified as having undergone parthenogenetic development, only 6 were produced by breeds of chickens other than Cornish and Cornish crosses. It is concluded that parthenogenesis, to a large degree, is under some sort of genetic control. (AH e3-19)

4. Homograft reaction and immunological tolerance. The graft versus host splenomegaly response in embryos is being utilized to study the effect of sex on histocompatibility in turkeys. In a first experiment, 0.3 ml. of adult male or female heparinized blood was placed on the chorioallantoic membrane of randomly selected 17-day turkey embryos. At 26 days incubation, all embryos were autopsied and their spleen weights recorded. The number of observations and mean spleen weights for the 4 donor-host combinations were: 21 male to male = 26.43 mg., 34 male to female = 26.27 mg., 34 female to male = 23.78 mg., and 22 female to female = 16.22 mg. Mean spleen weights for control embryos which received heparinized saline were: males 14.85 mg. and females 14.11 mg. The fact that the mean weight of all groups except female to female were considerably higher than the mean control weights suggested that the sex of either host or donor might influence histocompatibility. However, there were many embryos in each group which gave no indication of splenomegaly. In hopes of reducing the number of unresponsive embryos the experiment was repeated, this time using embryos from 9 pedigreed matings and injecting 0.05 or 0.1 ml. of blood directly into the chorioallantoic vein of 97, 19-day embryos. Controls received injections of heparinized saline. Splenomegaly occurred in only 18 of 36 embryos from three hens, while all embryos from the other 6 hens



were entirely unresponsive. The number of responding embryos was too small to support conclusions about differences between the 4 donor-host sex combinations. However, the fact that 10 embryos which received male blood had an average spleen weight of 292.82 mg. while the 8 which received female blood had an average spleen weight of 109.12 mg., indicated that the degree of splenomegaly may be related to the sex of the blood donor. The most interesting observations were (1) that embryos from only 3 of the 9 hens bore histocompatibility antigens different from either of the blood donors and (2) that of their 36 embryos, 18 developed splenomegaly and 18 did not. This 1:1 relationship suggests that the splenomegaly syndrome in turkeys may be controlled to a great extent by a single segregating histocompatibility locus. We expect to pursue this interesting lead in subsequent research. (AH e3-20)

Preliminary data indicate that cross-fertilization of Beltsville Small White turkey hens with Dark Cornish chicken semen may be blocked by prior intraperitoneal injections of the hens with the specific chicken semen. Fifteen virgin turkey hens - five groups of 3 full sisters - were placed in individual laying cages and brought into egg production in late January 1965. Two sisters of each triplet were to be injected later with Dark Cornish chicken semen and one sister of each triplet was to remain uninjected. Still later all were to be inseminated with semen from Dark Cornish males. Five different Dark Cornish males were used, each one assigned to a triplet as its specific semen donor for injection and insemination. Through 85 days preceding insemination a careful check was made of the incidence of parthenogenetic development in eggs laid by all hens. Those 10 to be injected laid 540 eggs, 76 (14.1%) of which showed parthenogenetic development after 9-11 days incubation. The 5 to remain uninjected laid 258 eggs, 44 (17.1%) of which showed similar parthenogenetic development. In the four weeks immediately preceding insemination two hens of each triplet received, twice each week, an intraperitoneal injection of at least 0.2 ml. Dark Cornish chicken semen in complete Freund's adjuvant.

Four days after the last injection all hens were artificially inseminated with at least 0.05 ml. undiluted semen. Seven identical inseminations followed at weekly intervals. In the 8-day period immediately following the first insemination, 3 of the 5 uninjected hens each produced one hybrid embryo which survived approximately 22 days incubation. No more were produced by these hens in spite of subsequent inseminations. No identifiable hybrids were produced by the injected hens throughout the total 63 days following the first insemination. During 33 days immediately following the first insemination, the combined incidence of hybrid and parthenogenetic embryonic development in eggs from the uninjected hens rose to 51.1% (47 of 92) while there was only a slight increase to 25.9% (51 of 197) in those from injected hens. In the final 30 days, development dropped to 18.7% (14 of 75) in eggs from uninjected hens and to 18.5% (29 of 157) in eggs from injected hens. Aside from apparently having inhibited hybridization of these two species by the specific intraperitoneal



injections, it was also interesting to note that cross-fertilization could not be maintained in the uninjected hens in spite of continued weekly inseminations. (AH e3-20)

5. Eggshell pigmentation in Japanese quail. The eggshell pigments of wild-type and mutant white-egg Japanese quail have been identified by spectrophotometric analysis of eggshell solutions, solutions of wild-type superficial pigment alone, and extracts of uterine tissue. Solutions of wild-type eggshells, without superficial pigment, in methanol - HCl (4:3) had optical density peaks at 380, 415, 560, and 680 m $\mu$ . Solutions of wild-type superficial pigment alone, in the same solvent, had optical density peaks at 415 and 560 m $\mu$ . Extracts of wild-type uterine tissue in 3N HCl showed optical density peaks at 410 and 560 m $\mu$ . Similar solutions of white eggshells had low optical density peaks only at 415 and 560 m $\mu$  and similar extracts of mutant uterine tissue had low peaks only at 410 and 560 m $\mu$ . Dissolution of eggshells in 3N HCl shifted the principal absorption peak from 415 to 410 m $\mu$  and extraction of uterine tissue in methanol - HCl shifted the same peak from 410 to 415 m $\mu$ . Red fluorescence of all extracts and solutions in ultraviolet light specifically indicated the presence of porphyrins. Comparison of these data with known optical density spectra indicated that both oöporphyrin (absorption maxima at 411 and 557.2 m $\mu$  in 25% HCl and 410 and 560 m $\mu$  in 3N HCl) and biliverdin (absorption maxima at 377 and 680 m $\mu$  in 5% HCl in methanol) were responsible for the pigmentation of wild-type eggshells. The slight pigmentation of white eggshells appeared to be due solely to small amounts of oöporphyrins since no biliverdin was detected on them or in mutant uteri. Thus the phenotypic difference between wild-type and mutant white-egg quail seems based on (1) a qualitative lack of biliverdin in white eggshells; and (2) a quantitative difference in the amount of oöporphyrin in and on the eggshell of both types. (AH e3-25)

Utilizing quail hens which tend to lay at the same hour each day in long uninterrupted sequences, it has been determined that superficial eggshell pigment deposition begins between the second and third hours prior to oviposition. At hourly intervals, beginning six hours prior to oviposition, such hens were autopsied and the presence or absence of superficial pigment on the eggshell surface of the uterine egg was recorded. Simultaneously, a 1 gram sample of uterine tissue from each of 3-5 hens was extracted and its optical density at 410 m $\mu$  was recorded. No superficial pigment was observed on 24 eggs removed from the uterus 4-6 hours prior to oviposition. Likewise, 12 of 17 eggs removed 3 hours prior to oviposition had no superficial pigment, although the remaining 5 did show slight darkening in the shell pores. By 2 hours prior to oviposition 13 of 13 eggs were superficially pigmented, 12 heavily and 1 moderately. Five of five eggs at 1 hour prior to oviposition were heavily pigmented. Similarly, there was an abrupt decrease in the mean optical density of uterine tissue extracts between the third and second hours, with the mean optical density at 2 hours prior to oviposition only 41.5% of that at 3 hours. (AH e3-25)

## B. Environmental Physiology

1. Photoperiod, fertility, and molt in turkeys. In connection with work on the influence of length of photoperiod on turkey reproduction, the molt of naturally mated Beltsville Small White toms was studied. Preseasonal molts of 20% or more seldom occurred in males not exposed to artificial light or exposed to an initial photoperiod of 11 hours, but occurred regularly in March, April, and May in males under 13- and 15-hour initial photoperiods started the previous December. However, there appeared to be no consistent relationship between these preseasonal molts and subsequent fertility. In only one of six groups of males under 13- and 15-hour photoperiods was preseasonal molt followed closely by a definite decline in fertility, although declining fertility was observed regularly following the "normal" summer molts occurring in June, July, and August. (AH e3-18)

2. Effect of light intensity on egg production. White Leghorn pullets hatched in March were maintained under 16 hours light daily. Supplemental light from 40-watt bulbs was supplied to all birds from hatching to about 20 weeks of age. Thereafter duplicate groups received supplemental light from 15-, 40-, 300-, 500-, and 1000-watt bulbs. Egg production differed little in hens under supplemental intensities afforded by 15- to 500-watt bulbs, but was lowered appreciably in groups receiving supplemental light from 1000-watt bulbs. (AH e3-23)

3. Light intensity and cannibalism. The effect of dim light on cannibalism was studied in White Leghorn hens confined in 24" x 18" cages, five birds in each. Two hundred hens in a "closed" house (wood walls, no windows) received light, 16 hours daily, from 15-watt bulbs. An additional 200 hens in an "open" house (walls of wire mesh) were exposed to daylight supplemented after sunset with light from 40-watt bulbs to give 16 hours illumination daily. Half the hens in each house were debeaked, half remained intact. The experiment was terminated at the end of 196 days because of failure of "foggers" in the open house. No deaths from cannibalism were recorded among debeaked hens in the closed house and only 2% among debeaked hens in the open house. Among the intact hens, losses from cannibalisms amounted to 15% in the closed house, 94% in the open house. The experiment will be repeated. (AH e3-23)



## PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Physiology of Reproduction

- Fraps, R. M. 1965. Ovarian feedback hormones in the hen's ovulation cycle. Proc. of the Second International Congress of Endocrinology, Part II, p. 1275. (Abs.)
- Fraps, R. M. 1965. Twenty-four-hour periodicity in the mechanism of pituitary gonadotrophin release for follicular maturation and ovulation in the chicken. Endocrinology 77, 5-18.
- Olsen, M. W. 1965. Twelve year summary of selection for parthenogenesis in Beltsville Small White turkeys. Brit. Poult. Sci. 6, 1-6. (AH e3-19)
- Opel, H. 1965. Timing of ovulation in chickens and Coturnix quail following oviduct transection. Anat. Record 151, 394. (Abs.) (AH e3-21)
- Opel, H. 1965. Oviposition in chickens after removal of the posterior lobe of the pituitary by an improve method. Endocrinology 76, 673-677. (AH e3-21)
- Opel, H. 1965. Failure of neurohypophysectomy to reduce egg shell thickness in chickens. Poultry Sci. 44, 1163-1164. (AH e3-21)
- Poole, H. K. 1965. Further evidence of heterozygosity in parthenogenetic turkeys. Nature 206, 324. (AH e3-20)
- Poole, H. K. 1965. Spectrophotometric identification of eggshell pigments and timing of superficial pigment deposition in the Japanese quail. Proc. Soc. Exptl. Biol. & Med. 119, 547-551. (AH e3-25)

Environmental Physiology

- Lowe, R. W. and Heywang, B. W. 1964. Performance of single and multiple caged White Leghorn layers. Poultry Sci. 43, 801-805. (AH e3-17)
- Marsden, S. J., McKee, G. S., and Crandall, M. C. 1965. Water deprivation and replenishment in poults. Poultry Sci. 44, 793-797. (AH e3-18)



POULTRY - NUTRITION  
Animal Husbandry Research Division, ARS

Problem. The goal of nutrition research is to amass information so that poultry diets may be formulated and fed to produce the best quality product at the least possible cost. The problem logically divides into two areas: (1) furnishing the nutritive requirements of poultry, and (2) the feedstuffs that supply these requirements. A refined methodology is needed to estimate more accurately the energy (carbohydrates and fats), protein (amino acids), vitamin and mineral requirements of poultry of various ages, strains, and levels of production. Even more urgently needed is information on the relationships that exist between these nutrients, if the formulation of optimum nutritive balance in diets is to be attained. Additional information is required on the effect of feed additives (antibiotics, arsenicals, hormones, enzymes, antioxidants, tranquilizers) on nutritive requirements, and on the utilization of protein and energy. Somewhere in the maze of requirements, interrelationships, and interactions, it must be determined which portion of the diet is for intestinal microorganisms and which is for the host. Also, the vast field of interrelationships between disease and nutrition remains to be explored. In the feedstuffs area, how much of a particular nutrient that is present should be known, but of more importance is how much is available to the bird. Thus, information on digestibility, absorption, chelation, and interactions is necessary. In addition, the complete composition of a feedstuff must be known. At the present, the proximate analysis is the only information available about major dietary constituents; consequently, the nutritionist does not know exactly what is being fed when a diet is formulated. There may be present growth promotant and/or inhibitors of which he is not aware.

USDA AND COOPERATIVE PROGRAM

This is a continuing program conducted by nutritionists on basic and applied research on the nutritive requirements and digestion and metabolism of poultry and the nutritive value of feedstuffs. The work is in progress at Beltsville, Maryland, and at the Southwest Poultry Experiment Station, Glendale, Arizona. Some phases of work at Glendale are carried on in cooperation with the Departments of Biochemistry and Poultry Science of the University of Arizona at Tucson.

A project to study malathion residues in poultry meat and eggs was contracted to Iowa State University of Science and Technology, Ames, Iowa. Its duration is for three years, 1965-1967.

Studies concerned with the influence of different factors on metabolism of vitamin A in chickens are in progress at the Hebrew University, Israel. Its duration is for five years, 1962-1967, and involves PL 480 funds. A project for the evaluation of the protein quality and energy values of feedstuffs available in India is in progress at Punjab Agricultural University, Ludhiana, India. Its duration is for five years, 1964-1969 and involves PL 480 funds. Investigations on calcium and phosphorus metabolism in chickens and factors influencing shell quality are in progress at the National and University Institute of Agriculture, Rehovot, Israel. Its duration is for five years, 1964-1969, and involves PL 480 funds.

The Federal effort devoted to research in the poultry nutrition area totals 6.3 professional man-years. Of this number 2.3 are devoted to digestion and metabolism, 1.6 to nutritive values of feeds, and 2.4 to nutritive requirements.

#### PROGRAM OF STATE EXPERIMENT STATIONS

Protein, energy, mineral, and vitamin requirements for maximum production and efficient feed utilization of chickens and turkeys are being investigated. Other aspects are concerned with changes in the nutritive requirement brought about by dietary nutrient interactions or interrelationships, genetic differences as related to nutritional needs, nutrition of mature hens to extend productive life and prevent reduction in production as age increases. Feedstuffs are being evaluated for nutrient availability, particularly amino acids. The effects of physical, enzymatic, and chemical treatment on nutrient availability are being studied. New antibiotics are being evaluated for growth promoting ability. Fundamental studies underway are designed to yield information on dietary factors controlling cholesterol biosynthesis, chemical structure of unidentified growth factors, trace mineral metabolism, particularly zinc, dietary components that effect the incidence of aortic rupture in turkeys, and the effect of anticoagulants and vitamin K on the incidence of blood spots in eggs.

Management practices that affect performance of laying hens, such as, nutrient and/or light restriction during the growing period, housing density in cage or floor pens, and controlled or natural environmental temperature are being studied. Broiler research is concerned with the effects of heat stress and housing density on growth rate and feed efficiency. The effects of bird density, litter condition, air movement and temperature control on rate of growth and reproductive performance of turkeys are also being studied.

The total State scientific effort devoted to poultry nutrition and management is 109.00 professional man-years.



## PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Nutritive Requirements

Linoleic acid studies were continued. Pullets were reared from hatch to maturity on the purified diet which has been used for several years to produce a severe linoleic acid deficiency. Starting at maturity they were fed the purified diet plus 4% linoleic acid. The egg production and egg size was about the same as that normally expected of pullets maintained on the purified diet plus 4% linoleic acid from hatching time. This showed that severe linoleic acid deficiency during the growing period did not affect subsequent performance. However, the production and egg size was significantly lower than that of pullets on a corn-soy diet containing 4% linoleic acid. This indicates that the purified diet is deficient in factors other than linoleic acid. Until these factors are recognized, it will be impossible to pinpoint the linoleic acid requirement of the hen with any degree of accuracy.

Studies on the effect of linoleic acid depletion on the fertilizing capacity of male chickens were continued. When three diets were fed, a purified diet devoid of linoleic acid, the purified diet plus 4% linoleic acid, and a practical diet containing 4% linoleic acid the average fertility was 62, 79, and 82%, respectively. Although the exact requirement of linoleic acid has not been determined, these results indicate that 4% is adequate for fertility. The decrease in fertility did not result from decrease in sperm motility or sperm count, thus metabolic changes in the sperm are indicated.

Studies with female Japanese quail indicate that they respond to a deficiency of linoleic acid in a manner similar to chickens. When quail were fed a purified diet devoid of linoleic acid, egg production dropped from 85 to 40% during the experimental period, whereas quail fed the same diet plus 3% linoleic acid, maintained production between 83 and 94% during this period. The average egg weight of the linoleic acid deficient quail was 16% less than that of the group receiving linoleic acid. Subsequent studies have indicated that 1 to 2% dietary linoleic acid is required for maximum egg production and 2 to 3% is required for maintenance of egg weight. (AH e2-20)

B. Digestion and Metabolism

1. Metabolism of fats. In studies to investigate further the effect of linoleic acid (18:2) on egg production, egg size, fertility, and hatchability, pullets were reared from hatching to 25 weeks of age on a purified diet practically devoid of 18:2. They were divided into six groups and



given safflower oil at increments to furnish zero, 10, 20, 40, 80, and 250 mg. of 18:2/bird/day, respectively. Increase in egg production, egg size, fertility, and hatchability paralleled the increase in dietary 18:2 with the exception that hatchability still remained zero at the 10 mg. level. Only 20 mg. of 18:2 daily were necessary to increase fertility from 73 to 92% and to decrease early embryonic mortality from 31 to 11%. Higher levels of 18:2 had no further effect on these reproductive traits. The presence of substantial quantities of eicosatrienoic acid (20:3) in plasma and yolk lipids of linoleic acid-deficient pullets suggests a relationship between the expression of reproductive traits and tissue level of 20:3. The level of 20:3 in the tissues decreased with each increase in 18:2, and these decreases were reflected by increases in egg production, egg size, and hatchability.

Experiments to study the relationship between the polyunsaturated fatty acids (PUFA) of menhaden oil and linoleic acid on the reproductive characteristics of the hen were conducted. Safflower oil was added to a purified diet devoid of linoleic acid to supply zero, 20, 80 and 1,700 mg. of linoleic acid/hen/day and menhaden oil was added to supply 20 and 80 mg. of linoleic acid/hen/day. The level of menhaden oil supplying 20 mg. of linoleic acid stimulated egg production, hatchability, and egg size to the levels obtained with safflower oil supplying 80 mg. of linoleic acid. This stimulatory effect of menhaden oil cannot be attributed to its linoleic acid content and is apparently due to the PUFA present in the oil. As in all previous experiments concerned with linoleic acid deficiency, the presence of eicosatrienoic acid in the tissues had an inhibitory effect on reproductive capacity. In this experiment the inhibitory effect was evident even in the presence of substantial quantities of essential fatty acids in the tissues. These results indicate that the PUFA in menhaden oil depress the synthesis of eicosatrienoic acid in the linoleic acid deficient hen and negate its effect on reproduction. (AH-e2-13)

2. Metabolism of calcium and phosphorus. Studies on the metabolism of these minerals at the National University, Rehovoth, Israel, show that the major calcium storage in laying hens is in the long bones. When hens were fed a low calcium diet, the ends and medullary segment of the femur lost a considerable amount of calcium during depletion. After three weeks on a replete diet, the initial content of the depleted segments was fully restored. Radioisotopes of calcium and strontium were administered to the hens and it was found that calcium deficiency increased the deposition of the radioisotopes in the eggshell. The data indicate the strontium-85 may be a good in vivo indicator for the movement of calcium. Other studies using radioisotope techniques indicated that most of the calcium absorption takes place in the proximal parts of the intestine. There was very little

absorption in the ileum and colon. In the duodenum there was considerable endogenous excretion of phosphorus. The percentage of calcium absorption was not influenced by the calcium content of the diet, but high dietary calcium depressed the absorption of phosphorus. The percentage calcium absorption was about twice as high during the periods of shell deposition than in periods when no shell was deposited. (A10-AH-20)

3. Utilization and function of vitamin A. Continuing studies concerned with vitamin A at the Hebrew University, Rehovoth, Israel, have indicated that when lutein is dehydrated to form anhydrolutein, a pro-vitamin A<sub>2</sub>, two inactive dehydration products are formed simultaneously. These products are in turn converted to anhydrolutein. The in vivo conversion of carotenoids to either vitamin A or A<sub>2</sub> was demonstrated by feeding tests with vitamin A-depleted mice. Studies on the effect of dietary protein levels and supplementation with thyroxine or uracil indicate the following: liver vitamin A depletion was reduced by lowering dietary protein level; protein malnutrition resulted in lowering vitamin A in alcohol form in the liver; depletion of vitamin A in liver was related to the percentage of the vitamin in alcohol form; thyroxine increased both vitamin A depletion from the liver and the percentage of vitamin A in alcohol form, whereas thiouracil had the opposite effect. Low protein levels significantly lowered vitamin A and protein in the plasma. Other studies indicated that the presence of enzymes which destroy carotene decreased the availability of carotene from green plants. Autoclaving improved the stability of carotene in plant tissue. (A10-AH-7)

#### C. Nutritive Value of Feeds

1. Effects of feeding cottonseed meal. Tests at Glendale in cooperation with the University of Arizona using four meals made from glandless seed and four meals made from glanded seed at 10 or 20% dietary level were conducted at two locations with laying hens. At one location the eggs were stored 15 days at 50-55°F. or three or six months at 35°F. At the other location all eggs were stored 14 days at 42°F. when some were examined and the remainder were stored at 32°F. for a total period of 4-1/2 or 6 months. When the glandless meals were fed at the 10% level, no pink whites were observed at any of the storage periods. At the 20% level one of these meals did not produce pink whites after six months' storage. Discolored yolks were observed with two of the four glanded meals after 14 days storage, and with all glanded meals except one after 4-1/2 and 6 months. Although there was an unexpected high incidence of yolk discoloration from the glandless meals the intensity of discoloration was relatively light. The data indicate that "bound" gossypol was liberated in the digestive tract of some layers.



Other studies at Glendale in cooperation with the University of Arizona showed that when two cottonseed meals, each containing less than 0.1% lipids, 0.02% free gossypol, and about 1% bound gossypol, were substituted at 10 and 20% levels for soybean meal, there was no appreciable egg discoloration. However, when 0.5% cottonseed lipids were incorporated in the diets, a higher amount of discoloration was produced. (AH e2-17)

2. Nutritive value of grains. In tests conducted at Glendale in cooperation with the University of Arizona, the nutritive value of five varieties of sorghum were compared with that of corn in chick diets. Three of the varieties were inferior to corn and two varieties were equal to corn. However, when the diet containing corn was equaled in protein content to the diet containing sorghum, then one of the superior varieties was found to be inferior to corn. (AH e2-15)

Tests were continued to determine the feeding value of corn, wheat, oats and barley. Each of these grains was fed at protein levels of 10, 12.5 and 15%. The diets were isocaloric for each protein level. The four grains were equal in supporting egg production at the 12.5 and 15% protein level. At the 10% protein level, oats was the only grain in both experiments that supported egg production equal to that of the higher protein levels. In the second experiment the production obtained with corn approached that obtained with oats. Feed conversion decreased as the protein level decreased, irrespective of the grain fed, but there was no difference between grains on the different protein levels. There was a positive correlation between protein level and body weight for all the grains. At each protein level the birds fed oats showed the greatest gains in body weight. This phase of the investigation has been completed. (AH e2-14)

3. Amino acid content of feedstuffs. Investigations on methodology of amino acid analyses have shown that the period of time that proteins are hydrolyzed affect the amino acid values obtained. It has also been shown that the enzymatic removal of starch from feedstuffs before hydrolysis prevents the partial destruction of some amino acids during hydrolysis and results in a more accurate estimation of the amino acid content. A new method has been developed for the precise and accurate estimation of tryptophan by chromatographic analysis. (AH e2-16)

## PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Nutritive Requirements

- Calvert, C. C. 1965. The performance of female Japanese quail on linoleic acid deficient diets. Poultry Sci. (Abs.) (In press). (AH e2-13)
- Lillie, R. J., Calvert, C. C. and Menge, H. 1965. The fertilizing capacity of the domestic cock as affected by linoleic acid. Poultry Sci. (Abs.) (In press). (AH e2-20)
- Lillie, R. J. and Denton, C. A. 1965. Effect of lighting systems in the grower and adult periods upon the overall performance of White Leghorns. Poultry Sci. 44: 809-816. (AH e2-14)
- Lillie, R. J. and Denton, C. A. 1965. Protein and energy interrelationships for laying hens. Poultry Sci. 44: 753-761. (AH e2-16)
- Kurnick, A. A., Heywang, B. W., Hulett, B. J., Vavich, M. G. and Reid, B. L. 1964. The effect of dietary vitamin A, ambient temperature and rearing location on growth, feed conversion, and vitamin liver storage of White Leghorn pullets. Poultry Sci. 43: 1582-1586. (AH e2-15)
- Reid, B. L., Heywang, B. W., Kurnick, A. A., Vavich, M. G. and Hulett, B. J. 1965. Effect of vitamin A and ambient temperature on reproductive performance of White Leghorn pullets. Poultry Sci. 44: 446-452. (AH e2-15)

Digestion and Metabolism

- Menge, H., Calvert, C. C., and Denton, C. A., 1965. Further studies of the effect of linoleic acid on reproduction in the hen. J. Nutrition 86: 115-119. (AH e2-13)

Nutritive Value of Feeds

- Kemmerer, A. R. and Heywang, B. W. 1965. Effect of the addition of cottonseed lipids to cottonseed meal on egg discoloration. Poultry Sci. 44: 889-890. (AH e2-17)
- Heywang, B. W. and Vavich, M. G. 1965. Discoloration in eggs from layers fed cottonseed meals from glandless and glanded seed. Poultry Sci. 44: 88-89. (AH e2-17)
- Kemmerer, A. R., and Heywang, B. W. 1965. A comparison of various varieties of sorghum as a substitute for corn in practical chick diets. Poultry Sci. 44: 260-264. (AH e2-15)



Heywang, B. W., Heidebrecht, A. A. and Kemmerer, A. R., 1965. Discoloration in stored eggs when layers at two locations were fed cottonseed meals made from glandless and glanded seed. Poultry Sci. 44: 573-577. (AH e2-17)

POULTRY - IMPROVEMENT OF VIABILITY  
Animal Husbandry Research Division, ARS

Problem. Leukosis continues to head the list of diseases that cause a high mortality among chickens. It is prevalent in both young and mature stocks, and on most, if not all, farms where chickens are reared. It is also responsible for high rates of condemnation at processing plants and poor performance with respect to growth and egg production.

The yearly financial losses to the poultry industry of the United States from leukosis mortality alone are estimated to be in excess of \$65,000,000. At this time when the margin between the cost of production and the price received for poultry products has been reduced to a very low figure, it is more urgent than heretofore that additional emphasis be placed on research directed towards the development of effective control measures for the leukosis complex. Also, such studies with chickens continue to be invaluable models for research on leukemia in other animals and man.

USDA AND COOPERATIVE PROGRAM

This is a continuing program in virology, pathology, immunology, epizootiology and genetics on basic and applied studies of the neoplasms of the avian leukosis complex. Most of the studies are conducted at the Regional Poultry Research Laboratory, East Lansing, Michigan. Work is also done in cooperation with more than a dozen other Federal, State, or commercial agencies located in several sections of the United States.

The primary objective of these studies is to develop a practical program for the prevention of losses due to the diseases of the avian leukosis complex. Most of the effort has been and currently is directed towards two approaches. The first is to develop a program to increase resistance of the chickens to avian leukosis. It has been found that the level of viral stimulated antibodies and certain yet undefined physiologic mechanisms are genetically controlled and have very important influences on resistance. For significant progress in this approach, it has become evident that basic studies must continue to be conducted on (1) mode of inheritance, (2) mechanism of gene expression, (3) interrelationships of resistance to different tumor viruses, avenues of exposure, and other genetically controlled traits, (4) dynamic interrelationship between infection, antibody, and neoplasms, (5) ultrastructure, biochemistry and the molecular biology of the causative virus and infected cells, and (6) the mechanism of influence of the bursa of Fabricius on neoplasia.

The second approach is directed towards the prevention of infection and/or elimination of the disease. Avian leukosis is a contagious disease. The infectious virus is transmitted not only by direct contact with infected chickens and with infected environment, but also via the infected embryonating egg. The most important prerequisite for progress in the



development of eradication measures is a simple but adequate method of detecting current or past infection. Only recently has notable progress been made on this aspect and further simplification of procedures can be expected. Such fundamental epizootiological information as the extent and prevalence of infection, the modes of spread of infection, the importance of various vectors and reservoirs, the importance of various environmental factors and other concomitant diseases, and the influence of passive as well as active immunity must be obtained before a rational program of eradication can be developed.

Recent developments have demonstrated that there are at least two different families of viruses--not one, as formerly thought--that cause similar types of leukoses. This emphasizes the magnitude of the problem that still faces us.

Research contracts, each for a three-year period, are in effect with Cornell University, the University of Connecticut, and the University of Georgia. In addition, cooperative agreements with at least seven universities or commercial concerns were in effect during the reporting period. Also cooperating in this area of research are the National Institutes of Health, the American Cancer Society, and the Animal Health Division, and the Animal Disease and Parasite Research Division of the Agricultural Research Service.

The Federal scientific effort devoted to the research in these areas during the past year totaled 11.3 professional man-years. Of this number, 2.4 were devoted to in vivo and in vitro studies of the causative agent, 3.0 to immunology and serology, 3.2 to epizootiology, 1.7 to genetics, and 1.0 to biophysics and biochemistry.

#### PROGRAM OF STATE EXPERIMENT STATIONS

The State agricultural experiment stations are conducting research into genetic, physiological, and nutritional factors affecting the incidence and severity of the avian leukosis complex. Blood group antigens are determined in several populations of domestic fowl varying in degree of resistance and susceptibility to the disease. Relationships between blood antigens and disease resistance are explored. Other research utilizes select viruses of the leukosis complex in an attempt to determine the mode of gene action involved in resistance to the disease. Several serum enzymes are under investigation in populations bred for differences in resistance to leukosis. Also, the effects of protein, vitamin and mineral levels in feed and their relationships to leukosis are examined.

The total State scientific effort devoted to Improvement of Viability in poultry is 2.0 professional man years.



## PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. In vivo and in vitro Studies of the Causative Agent

The standard stocks of Rous sarcoma virus have for helper viruses two leukosis viruses identified as RAV-I and RAV-II. These correspond to types A and B respectively of the leukosis virus groups by Dr. Vogt. It was found that RAV-II causes visceral lymphomatosis and osteopetrosis in both inbred line 7 and line 15I. Whereas RAV-I like RPL 12 causes these neoplasms only in line 15I chickens. Erythroblastosis occurred only in line 15I and was caused by both viruses.

These and other data conclusively demonstrate that within the RIF positive group of viruses which cause visceral lymphomatosis (lymphoid leukosis), osteopetrosis, other leukosis and tumors, there are at least 2 distinct subgroups which are separable on the basis of (1) antigenic differences and (2) susceptibility to infection as determined by different gene loci. These findings further emphasize the importance of the use of experimental hosts of known susceptibility to the various viruses or subgroups of viruses under investigation.

Further studies on the role of the bursa of Fabricius in the causation of visceral lymphomatosis have revealed that this malignancy is exclusively of that component of the lymphoid tissue that is derived from and/or dependent for its development upon the bursa of Fabricius. The studies further exclude the thymus or the thymus dependent lymphoid tissue from participation in the pathogenesis of visceral lymphomatosis. Bursectomy can be performed as late as 5 months after viral inoculation and still influence the development of lymphoid tumors. Antibody production, a function of the bursa-dependent lymphoid tissue, is also demonstrated to be impaired early in the development of visceral lymphomatosis.

It has been known that the application of the male sex hormone to the fertile egg either by direct injection or dipping in an alcoholic solution, has a marked effect on the development of lymphoid tissue. In one experiment, one-half of the chicks hatched of fertile eggs that had been dipped in a solution of testosterone had no bursal tissue at necropsy. Of 96 such chickens only one developed visceral lymphomatosis whereas 75% of 86 chickens which had intact bursas, whether or not they were from dipped eggs, developed visceral lymphomatosis. These results point to a chemical means of preventing the development of visceral lymphomatosis, however, this cannot at this time be considered a practical method since the dipping had a marked adverse effect on hatchability. (AH e6-10)

Attempts are being made to establish continual cell lines propagated in vitro from chicken embryos of pedigree matings of inbred lines 15I and 7. Such cell lines will insure genetic continuity and provide a highly reproducible in vitro system for critical studies of the viruses of the avian leukosis complex. Currently, three separate cell lines of 15I and two



separate cell lines of line 7 cells are in continual passage. Line 15I cells are in their 7th, 14th, and 33rd passages. Line 15I cells of the 7th passage gave a Rous sarcoma virus (RSV) titer ( $2 \times 10^7$ ) similar to 1st or 2nd passage cells. The 14th and 30th passage cells gave titers of  $1 \times 10^5$  and  $5 \times 10^4$  cc respectively. Microscopically, cells obtained after the 10th passage appeared more elongated than those of the first few passages, however, morphologic appearance does not indicate transformation. Line 7 cell lines are in the 8th and 13th passages. Both cell lines at the 7th passage were tested for RSV growth. Titers for both were low, i.e., less than  $1 \times 10^2$  cc. The criteria set by this laboratory for an established cell line is 100 passages or one year in continual culture.

Analysis of human sera from individuals in close contact with poultry has been carried out. Results indicate the presence of anti-RSV activity and a chick embryo cell toxicity factor in the sera. Because of the very high proportion of the samples exhibiting these effects, investigations into the details of collecting and processing the sera were carried out. It was shown that the commercial blood collecting "vacutainers" were contributing to this effect. Types tested included those with red, non-toxic pink, and blue stoppers. In addition, regularly used glass crew-cap test tubes and Erlemeyer flasks were employed. All cell culture systems exposed to sera from those vacutainers with the colored stopper showed evidence of toxicity. (AH e6-24)

The biocharacteristics of the JM strain of leukosis virus, a strain selected as a prototype for RIF-negative leukosis viruses which induce Marek's disease and "acute leukosis" have been studied in line 7 chickens. Transmission of JM virus was routinely successful when cellular virus preparations were given to one-day-old chicks and when uninoculated chicks were raised in direct or indirect contact with JM-inoculated chicks. Transmission with cell-free virus preparation was successful in one of six trials.

Chicks exposed to JM virus by inoculation or direct contact usually developed typical Marek's disease with clinical lameness and paralysis in two to five weeks. The initial deaths in inoculated birds occurred in 18-19 days with a median days-to-death of 28-30 days. Mortality during a 70-day-period for inoculated and contact-exposed birds averaged about 85 and 52%, respectively. These response levels could be increased appreciably by the inclusion of survivors with lesions.

Microscopic lesions occurred before 12 days in inoculated birds and consisted of infiltration of peripheral nerves and spinal ganglia with lymphocytes and plasma cells. A few contact-exposed birds also developed minor lesions in the same interval of time.

Most birds at death had enlarged nerves and about 50% of birds positive by gross necropsy had visceral tumors, principally of the gonad.



Sex did not influence the response to JM virus. Detectable age resistance occurred within four weeks. Potency was largely lost by freezing (rapid or slow). Attempts to propagate the virus in chick ovarian cells have thus far been unsuccessful. The rapidity and ease with which transmission by direct or indirect contact occurs has pointed out the absolute necessity for rigid isolation (isolator cages) for transmission and virus assay trials. (AH e6-30C)

## B. Immunity and Serology

The interrelationships between different ages of the host at the time of exposure by different routes to various doses of the RPL 12 leukosis virus and the neoplastic as well as antibody responses were studied in line 151 chickens.

Intravenous inoculation of 10-day embryos or day-old chicks resulted in high early neoplasm mortality with only a few chickens developing antibody. Most of the survivors had a persistent viremia. The latter no doubt was due to an immune tolerance to the virus resulting from infection occurring while the chick was immunologically incompetent. When infection was delayed to 2 or 4 weeks of age, most chickens developed antibodies and died with neoplasms in a variable period depending on dose and route of inoculation. Most chickens inoculated at 16-28 weeks of age developed antibodies, but only a few died of neoplasms. All chickens inoculated intravenously at one year of age developed antibodies, but none developed neoplasms. It would appear that a certain minimum effective dose of virus is required to stimulate the formation of antibodies irrespective of age. A large dose is required to induce neoplasms; in addition, as the chickens become older there is a decrease in susceptibility to formation of neoplasms. The intravenous route is more efficient than the intra-nasal route for induction of infection resulting in either antibody formation or neoplasms.

Comprehensive studies were conducted on the antigenic characterization of several Rous sarcoma viruses (RSV) including RSV (Bryan), RSV (B-high titer), RSV (B-RPL 22), RSV (CT 916), RSV (Harris) and RSV (Schmidt-Ruppin) and RSV to which had been attached the leukosis strains RPL 12, RPL 28, RPL 29, RPL 35 and BAI strain A. All appear to be antigenically related with the possible exception of RSV (Harris). Those leukosis or sarcoma virus strains which originated from RSV (Bryan) appear to be identical antigenically. The two field isolates RPL 28 and 29 are similar to RPL 12 virus. BAI strain A virus is antigenically very complex. Its antiserum neutralizes nearly all viruses tested, but it is neutralized only by its homologous antiserum.

In the course of conducting complement fixation tests for avian leukosis viruses (COFAL), it was observed that some apparently normal embryos and/or cells cultured in vitro reacted strongly in the test. Studies to elucidate the cause of these reactions indicated that genetic factors played an



important role in the presence of this antigen. Based on tests of embryos and culture cells, only certain sire-dam matings resulted in progeny producing a high concentration of this reactive material. Experiments are in progress to determine whether or not a virus, related to the avian tumor viruses, is involved.

Preliminary results of a procedure employing a combination embryo inoculation and the COFAL technique indicate that a very sensitive, rapid assay for strain RPL 12 and/or other leukosis viruses may be developed. This possibility is being investigated further. (AH e6-17)

### C. Epizootiology

In cooperative studies with the National Institutes of Health, the occurrence of lymphomatosis and the presence of RIF positive leukosis virus and antibody was followed in samples of commercial and 15I chickens on two commercial farms in Indiana. Farm 1 experienced a relatively high rate of mortality from lymphomatosis and Farm 2 a relatively low rate.

Analyses completed to date indicate the following findings or relationships:

- (a) Maternal antibody in 1-day-old chicks was present in about 1/2 of the chickens of both farms. By 10 weeks of age none had antibody. Line 15I chickens had no maternal antibody.
- (b) Acquired antibody at 6-20 weeks of age was found in about 40% of chickens on both farms, however, among the 15I chickens in contact with the commercial chickens, the number with acquired antibody on Farm 2 was twice that on Farm 1.
- (c) Viremia in day-old commercial chicks due to congenital infection was about 3 times greater in Farm 1 than in Farm 2. At ten days of age the percentage was less than at one day indicating a natural reduction of the congenital infection. The line 15I chickens had no infection at one day, but by 10 days almost 1/2 were viremic. The proportion was higher among those exposed to Farm 1 chickens than those exposed to Farm 2 chickens.
- (d) Viremia measurements of plasma taken at 6, 14, and 20 weeks of age showed that on the average somewhat less than 1/2 of the commercial chickens and about 2/3 of the 15I chickens had a viremia. The proportion on Farm 1 was 1.5X higher than on Farm 2.
- (e) Neoplastic mortality was about 3X higher on Farm 1 than on Farm 2.

Differences in the level of infection as indicated by the percentage with viremia, but not by percentage with antibody, appear to be positively

related to the neoplastic mortality in the 2 flocks studied. The higher neoplastic mortality and infection level in Farm 1 may have been due to the much higher congenital infection as evidenced by viremia at 10 days of age.

In attempting to interpret the results of this study, one must consider the possibility that some or even most of the lymphomatosis mortality in this study was not due to the RIF positive viruses which are the only ones detectable by the methods used. (AH e6-27)

#### D. Biochemical and Biophysical Studies

Rous sarcoma virus preparations have been radio-labeled with tritium. Phenol extraction procedures were used. Low background radio counts indicate that most of the tritium was attached to the nucleic acid of the virus. The nucleic acid fraction gave counts of 300-500/min. whereas those of the protein "coat" were 50 or less. Chicken embryo cell cultures were infected with the radio-labeled virus. Cultures were harvested at various intervals and will be studied with the electronmicroscope using autoradiographic techniques. (AH e6-31)

#### E. Genetic Studies

Studies of the single gene locus influencing in vivo and in vitro susceptibility to RSV (Bryan) have been extended. It has been shown that this locus has a large effect on susceptibility to erythroblastosis induced by the RPL 12 strain of leukosis virus which is antigenically related to RSV (Bryan). Also homozygous resistant (rs rs) chickens do not produce as much specific antibody after inoculation with RPL 12 as the susceptible chickens (RS RS, Rs rs). This is presumably because the virus does not grow sufficiently in resistant chickens to provide enough viral antigen to invoke an immune response.

The influence of this locus on susceptibility to lymphomatosis under field conditions is under investigation. A second locus influencing susceptibility to other strains of RSV and related leukosis viruses has been suggested by work at other laboratories. Our preliminary data agree with this. Work is now underway to confirm this finding, and to determine the influence of both loci on susceptibility to several strains of leukosis virus.

Studies evaluating inoculation procedures as methods of selection for resistance to field exposure to leukosis have been continued for another year and extended to include studies of resistance to inoculation and contact exposure to isolates of acute leukosis virus. These studies suggest that susceptibility to acute leukosis is independent of susceptibility to chronic types. (AH e6-29)



## PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

In Vivo and In Vitro Studies of Causative Agent

- Burmester, B. R. and Frederickson, T. N. 1964. Variacion de la capacidad oncogenica de los virus productores de tumores en las aves. Acta Oncologica 3: 43-55. (AH e6-10)
- Burmester, B. R., Purchase, H. G. and Peterson, R. D. A. 1964. Lack of visceral lymphomatosis in bursectomized inoculated chickens. Poultry Sci. 43: 1306 (Abs.). (AH e6-10)
- Frederickson, T. N., Burmester, B. R. and Okazaki, W. 1965. Transmission of virus from field cases of avian lymphomatosis. II. Development of strains by serial passage in line 15I chickens. Avian Diseases 9: 82-103. (AH e6-10)
- Frederickson, T. N., Purchase, H. G. and Burmester, B. R. 1964. Transmission of virus from field cases of avian lymphomatosis. III. Variation in the oncogenic spectra of passaged virus isolates. Nat. Cancer Inst. Monograph 17: 1-29. (AH e6-10)
- Purchase, H. G. 1965. Rous sarcoma and its helper viruses. (A review). Avian Diseases 9: 127-145. (AH e6-24)
- Sanger, V. L., Frederickson, T. N. and Morrill, C. C. 1964. Measurement of bone growth in osteopetrosis. Can. J. Comp. Med. Vet. Sci. 28: 236-238. (AH e6-10)
- Ziegel, R. F., Burmester, B. R. and Rauscher, F. J. 1964. Comparative morphologic and biologic studies of natural and experimental transmission of avian tumor viruses. Nat. Cancer Inst. Monograph 17: 711-731. (AH e6-10)

Immunology and Serology

- Burmester, B. R. and Okazaki, W. 1964. Discussion of neutralization by antibody of strain RPL 12 and Rous sarcoma (Bryan) viruses as measured by different methods. Nat. Cancer Inst. Monograph 17: 509-522. (AH e6-17)

Genetic Studies

- Crittenden, L. B., Okazaki, W. and Reamer, R. 1964. Genetic control of responses to Rous sarcoma and strain RPL 12 viruses in the cells, embryos and chickens of two inbred lines. Nat. Cancer Inst. Monograph 17: 161-177. (AH e6-29)

Crittenden, L. B., Purchase, H. G. and Okazaki, W. 1964. Genetic analysis of responses to artificial and natural exposure to avian tumor viruses. Poultry Sci. 43: 1310 (Abs.). (AH e6-28)



POULTRY - BROILER LOSSES  
Animal Husbandry Research Division, ARS

Problem. It is necessary to determine the causes of the continuing losses from condemnations - a major problem of the broiler industry. The chronic respiratory disease complex, commonly referred to as CRD or air sac disease is one of the principal causes of condemnations. The presence or absence of pathogenic strains of the pleuropneumonia-like organism, Mycoplasma gallisepticum, largely determines whether chickens will develop air sac disease in the presence of Newcastle disease, infectious bronchitis, or secondary invaders, such as E. coli. Knowledge is needed of the fundamental principles regulating the behavior of the organisms associated with the CRD complex and the host response to them. The great range in host response indicates that genetic variation, nutrition, environment, and management play a part in the severity of the response of individual flocks of chickens. Additional basic information is needed on the effect of stresses on the physiological well-being of the broiler chicken. It is of practical importance to know more of the poultry housing and management factors which adversely affect the bird.

USDA AND COOPERATIVE PROGRAM

A basic and applied program of research directed toward the reduction of losses from broiler condemnations is conducted jointly by specialists in agricultural engineering, animal diseases, poultry management, genetics, biochemistry, and physiology. Two locations are involved in this work, the Southeast Poultry Research Laboratory, Athens, Georgia, and the South Central Poultry Research Laboratory, State College, Mississippi. The Animal Husbandry Research Division's work at Athens emphasizes genetics and physiology in relation to the chronic respiratory disease complex and the work at State College emphasizes environment, management, and nutrition in relation to condemnation losses.

This research program is cooperative with the Animal Disease and Parasite and Agricultural Engineering Research Divisions, ARS. Local cooperation of State experiment stations and the broiler industry in the southeast and south central regions is an important part of the program, particularly with respect to field trials.

The Federal effort devoted to research in this area totals 3.4 professional man-years. Of this number 1.6 is devoted to management practices, 1.0 to genetic control, and 0.8 to environmental physiology.

PROGRAM OF STATE EXPERIMENT STATIONS

Effects of environmental factors on broiler losses are studied at various State agricultural experiment stations. Different materials used in litter

are under investigation. Scientists are developing improved methods of handling and transporting live broilers. Other experiments are concerned with temperature, humidity, ventilation and light in relation to broiler house design.

The total State scientific effort devoted to Environment as related to broiler losses is 8.0 professional man-years.

## PROGRESS -- USDA AND COOPERATIVE PROGRAMS

### A. Genetic Control

In cooperation with the Animal Disease and Parasite Research Division, methods of challenge with Newcastle disease virus (NDV) were investigated to develop an objective technique for identification of resistant and susceptible individuals within the Athens Randombred subpopulation maintained at the Southeast Poultry Research Laboratory.

In embryo-inoculation trials, a mean flock titer to NDV-GB strain for a random sample of 16 replicates was  $10^{-8.3}$ . Titers were then determined for 75 individual hens in 8 single male matings at the  $10^{-8}$  and  $10^{-9}$  dilutions. Of these, 64 were tested twice and each scored for titer above and below the population mean.

A second series of tests was made on 2-week-old pedigreed chicks from 64 hens, after determination of the mean response by challenge of random groups. In two trials, exposure was by contact with chicks which had been inoculated intratracheally. In a third trial, each chick was inoculated intramuscularly with 0.1 ml. of an  $LD_{50}$  dilution of NDV.

Repeatability of results from the same dam across all tests (embryo challenge and chick challenge) was low ( $r = .24$ ). This is not surprising, considering the wide range of phenotypes for color (and presumably genotypes) found in chicks from the same dam in this population.

Initially, sons and daughters of 11 dams, and daughters only of six others, which showed most consistent evidence of resistance by the above tests were saved for reproduction of the  $F_1$  generation of the resistant line. For the susceptible line, sons and daughters of 12 dams, and daughters only of seven others, were saved.

Sons from dams in both susceptible and resistant lines were sacrificed, adrenals and bursae weighed, and blood samples taken to study possible physiological relationships to disease resistance. (AH e7-2)

Two trials were conducted to check the efficacy of polyvinylidene chloride co-polymer resin films (Cryovac) on hatchability of eggs. Eggs stored at  $52^{\circ}\text{C}$ . for three weeks in cartons wrapped in the film hatched 12% better than those stored similarly but without wrapping. This was statistically



significant. In another trial pedigreed eggs from 60 ARB hens were stored for periods from 1 to 4 weeks with and without film wrapping. Two to three fresh eggs per hen were used as controls. Hatchability of fertile eggs was 66.9, 83.3, and 85.3% for the unwrapped, wrapped, and controls, respectively. Although detailed statistical analysis is not complete, wrapping in film improved hatchability of certain hen's eggs more than others. When large numbers of eggs per hen are required in the same hatch, the use of Cryovac-type wrapping appears beneficial.

## B. Management Practices

On-the-farm studies of avian nephrosis (gumboro disease) in broilers were completed. The studies show that a curve of mortality is present in a typical outbreak which can be used with clinical manifestations, and with or without the lesions of the disease, as a valuable tool in the diagnosis of the disease. The mortality records involving 2,060,258 chicks started in 209 houses on 28 infected farms showed that the symptoms first appeared at about 25 days of age and lasted about 7.5 days. Mortality usually peaked half way through the active period. The average total mortality was 8.8%, of which 5.6% occurred during the period when avian nephrosis was active. Previous to these studies poultrymen used antibiotics, vitamins, sulfa drugs, and molasses in the drinking water and used detergents, synthetic phenolic disinfectants, quaternary ammonium compounds and lye, with and without litter changes, in an effort to prevent, control, or eradicate the disease. These studies indicated that none of these practices are consistently successful in reducing gumboro disease. (AH e7-1)

Field trials were conducted to determine the effects of feeding a commercial broiler ration supplemented with a dried culture of live Bacillus megatherium. In one trial involving 43,409 chicks the mortality was 7 and 6% respectively for the treated and control groups. In another trial of 50,835 chicks the mortality was 7.5% in the treated group as compared to 4.0% in the controls. (AH e7-1)

Observations were made at constant room temperatures of 70°F. and 40°F., and at 70°F. and 60°F. To date, the trend is for a slightly heavier weight at 70°F. than 40°F., especially during the early stages of growth, but no difference at 70°F. and 60°F. Feed efficiency was always better at the higher room temperature. Comparisons have also been made at these room temperatures between canopy hovers 74 and 99 inches in diameter. There were no marked differences in growth or feed efficiency between the two hovers although the trend was better feed conversion with the 99 inch hover at 40°F. From these data it appears that a constant temperature of 40°F. is not a serious stress in the presence of good management.

There were no consistent differences in growth, feed conversion, livability, or percent condemnation between broilers raised in insulated and non-insulated pens, canopy and infra-red hovers, or when heat was discontinued at three weeks (May, June, July and August). During hot weather there

were no marked differences to six weeks of age between broilers raised in insulated or non-insulated pens, with or without floor fans, or with 0.8 or 1 square foot of floor space per bird. (AH e7-4)

### C. Environmental Physiology

A total of 775 pedigreed male chicks from 32 sire families of Athens Randombred stock were screened for their responses at three ages to exogenous administrations of adrenocorticotropin and hydrocortisone. Autopsy data, which included adrenal and bursa of Fabricius weights, have been collected on all birds. Data are currently being analyzed to determine familial differences in the effects of those hormones considered important in the stress response. (AH e7-3)

## PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

### Management in Relation to Condemnations

Parkhurst, R. T. 1964. On-the-farm studies of Gumboro Disease in broilers. Avian Diseases, 8: 584-596. (AH e7-1)

Parkhurst, R. T. 1965. Field experience with Gumboro Disease in broilers. Poultry Sci. (Abs.) (In press) (AH e7-1)



**POULTRY PERFORMANCE AND MANAGEMENT RECORDS**  
**Animal Husbandry Research Division, ARS**

Problem. Effective livestock and poultry improvement cannot be accomplished effectively without adequate records of performance and management. In order to have widespread utility, these records must be produced through carefully coordinated programs yielding uniformity in measurements, standardized analytical procedures, and accuracy and integrity in animal identification and genealogy. Continual revision of uniform data collection, reporting and evaluation techniques in accordance with current research findings requires integration of program operations and research. Only in this manner can there be a continual chain of discovery, field testing, and application with the needed genetic and environmental improvement.

**USDA AND COOPERATIVE PROGRAM**

This is a continuing long-term program of performance testing dairy cattle and poultry, including the evaluation of the genetic merit of dairy cows, sires and herds, chickens for egg or meat production, and turkeys. Also included in the program is the control of egg-transmitted poultry diseases. The work on dairy cattle performance testing is cooperative with 50 States and Puerto Rico and the Records and Breeding Committees of the American Dairy Science Association. Cooperation is also carried out with the National Association of Animal Breeders and the various dairy cattle breed registry organizations. The poultry work is cooperative with Official State Agencies in 47 States and with the supervisors of 25 random sample tests in the United States and Canada.

The Federal scientific effort devoted to the programs in this area totals 7.0 professional man-years. Of this number, 5.0 are devoted to dairy cattle performance testing and 2.0 to poultry performance testing.

**PROGRAM OF STATE EXPERIMENT STATIONS**

The effort of the State stations in this area is quite large. It is difficult, however, to make a manpower estimate which would be distinct from that in breeding sections. As the USDA material indicates, much of the effort in DHIA and in poultry testing is cooperative with the States. At several State stations, DHIA records are processed for dairymen on a reimbursable basis. Data derived is used in estimation of genetic parameters, etc., by resident investigators. Similarly, the random sample poultry tests on broiler and egg production strains conducted at State locations provide information on performance and mortality of commercial stocks. Eggs and meat from these poultry tests are used in detailed studies such as strain comparison of chemical constituents and correlations of these items with production traits.

Animal performance and management records with beef cattle, swine, and sheep are frequently obtained through cooperation with producers, extension service, and industry. In several States, swine testing stations have been established and operated on a self-sufficient basis supported by fees. Research personnel often act in an advisory capacity. In many States, programs of on-the-farm performance and progeny testing of beef cattle have been developed. Again, research and extension people frequently cooperate in this endeavor. At central facilities in some States, bulls belonging to producers are tested for growth and feed efficiency often under the direction of research personnel. Similarly, a limited number of ram testing stations have been developed for indicating growth rate.

No estimate of State station professional man-years is made.

## PROGRESS -- USDA AND COOPERATIVE PROGRAMS

### Poultry

1. National Poultry and Turkey Improvement Plans. Over 14.4 thousand flocks containing 32.9 million breeding chickens and 1.9 thousand turkey flocks containing 3.7 million birds were officially classified under National Plan standards. All flocks qualified for a disease control classification and 94.4% of the chickens and 97.3% of the turkeys were also classified under one of the breeding phases of the Plans. These birds produced hatching eggs for 1,418 chicken hatcheries with incubator capacity of 316 million eggs, and 325 turkey hatcheries with capacity for 52.2 million eggs.

More than 16.8 thousand prospective breeding flocks containing 37.0 million birds were tested for pullorum disease and fowl typhoid. The number of reactors to the initial blood test reached an all-time low for both chickens and turkeys. The percentage of chicken reactors dropped from .0055 to .0044, while turkey reactors declined from .0047 to .0024%. Cooperating diagnostic laboratories reported 277 isolations of pullorum and typhoid organisms. Approximately one-half of these cases were followed up with field investigations by cooperating State agencies. With the continued cooperation of the diagnostic laboratories and State agencies, it is expected that increasing numbers of these infections will be traced to their sources and eliminated as potential disseminators of these two diseases.

Cooperating laboratories report the diagnosis of paratyphoid as well as pullorum and typhoid. During recent years the reports show a steady decline in pullorum-typhoid, while the incidence of paratyphoid has increased rapidly. In 1964, laboratories reported 1,108 cases of paratyphoid, as compared to 397 in 1960. These reports have shown *S. typhimurium* to occur frequently in turkeys. A program for the control of *S. typhimurium* was incorporated in the NTIP late in 1964. The laboratory reports also indicate a rapid increase in the incidence of paracolon infection during the last few years. As yet, no organized program for the specific control of paracolons has been adopted.



Provisions for a *Mycoplasma gallisepticum* (PPL0) control program in the Turkey Plan became effective in November 1964. However, participation in the program was limited until an approved antigen for conducting the blood test became available early in 1965.

The trend toward greater use of cross-mating in chick production continued during 1964-65. More than 3/4 of all chickens in participating flocks were in cross-matings. Conversely, less than 2% of the turkey breeding flocks were cross-mated. The growing acceptability of the large white turkey is reflected in 1964-65 participation reports, which show that for the first time, the number of large white turkeys exceeded the number of Bronze.

Reports of exports of hatching eggs, chicks, and poults show that approximately 10% fewer units were shipped to foreign countries in 1964 than in 1963. However, exports of products to be used for breeding purposes were 30% greater in 1964 than in the previous year. (AH e5)

2. Random sample performance tests. Data from 17 United States and 4 Canadian random sample egg laying tests were submitted to the Poultry Improvement office in 1964. The 1963 and 1964 data were combined into a two-year summary. This combination of data over the two-year period permitted comparison of the performance of 106,397 laying hens in 1,582 pens, and 143 different stocks tested at 63 locations in the United States and Canada. The combining of data obtained over a two-year period permitted more reliable predictions of the performance of stocks than the use of a single year's data. The results of these computations, expressed as regressed means, are predictions of what the performance of a stock would be, had it been tested at all 63 locations over the two-year period. These regressed means, along with the statistical significance of differences, expressed as 80% confidence limits, were computed for 16 economic traits of each stock tested.

The supervisors of four Random Sample Turkey Tests submitted records on 4,200 individual turkeys, representing 42 entries of 32 different stocks that were tested in 76 pens. These data were analyzed separately by tests, and Duncan's Multiple Range Test was applied to 12 traits to show the statistical significance of difference between entries within a test. (AH e5)

## PUBLICATIONS - USDA AND COOPERATING PROGRAMS

Poultry

Breed Distribution of NPIP Participating Flocks by States and Divisions, 1952-53, 1962-63, and 1963-64. ARS 44-2.

Tables on Hatchery and Flock Participation in the National Poultry Improvement Plan by States and Divisions, 1962-63 and 1963-64 and U. S. Summary 1958-59 to date. ARS 44-3.

Tables on Hatchery and Flock Participation in the National Turkey Improvement Plan by States and Divisions, 1962-63 and 1963-64 and U. S. Summary 1958-59 to date. ARS 44-4.

Hatcheries and Dealers Participating in the National Poultry Improvement Plan. ARS 44-6.

Annual ROP and Performance Test Summary 1963-64. ARS 44-7.

Participants in the National Turkey Improvement Plan. ARS 44-8.

Turkeys in NTIP Flocks and Their Distribution by States and Varieties, 1952-53, 1962-63, and 1963-64. ARS 44-11.

Turkey Performance Tests. 1964. ARS 44-13.

1964 Report of Egg Production Tests, United States and Canada. ARS 44-79-5.

Report of National Plans Conference, July 8-10, 1964. in Washington, D. C.



PRODUCTION INFLUENCES ON POULTRY PRODUCTS  
Animal Husbandry Research Division, ARS

Problem. Beef, lamb, pork, and poultry are excellent sources of wholesome and digestible animal proteins and fatty acids necessary in maintaining a healthy, appetizing diet. However, these meats must be of high quality, as well as in plentiful supply, if they are to retain their high position and esteem in the minds of consumers. Proper finish, a high proportion of lean, with adequate intramuscular fat, tenderness, full flavor, and color desired by the consumer are the goals the meat producer must strive to attain through breeding, feeding, and management. The quality of cuts and kind of meat are directly reflected in the demand and in the price of the product.

Egg shell strength and yolk quality, strength of wool, fatness, quantity, flavor, color, and tenderness of meat are all known to be influenced by production practices. However, these quality characteristics and many more are not well understood, even though they are of considerable economic importance. Effective measures of evaluating quality differences are of great importance in determining the nature and effect of production practices on the products.

USDA AND COOPERATIVE PROGRAM

This is a continuing program conducted by food product technologists, wool and fiber technologists, biochemists, chemists, physiologists, statisticians, and animal husbandmen engaged in both basic and applied research designed to develop methods and information which will be useful in evaluating quality and quantity of animal products and will be useful in aiding and directing livestock production. Research on beef, veal, lamb, and pork is directed at the influence of selection and breeding, nutrition, physiology, management, and other production variables on carcass and meat quality and quantity. Standards are being applied and adapted for appraisal of slaughter animals, of carcasses, and of meat cuts. The objective of the work with poultry and eggs is to ascertain those factors of nutrition, breeding, and management which contribute to the initial quality of poultry products and their capacity to retain that quality. Studies with wool, fur, and fiber are conducted to determine the physical, chemical, and biological structures and properties of wool and other animal fibers as influenced by production factors. The work is conducted at Beltsville, Maryland; Dubois, Idaho; Fort Wingate, New Mexico; Glendale, Arizona; and in cooperation with four State experiment stations. Cooperation is also carried out with the Eastern and Western Utilization Research and Development Divisions, the Human Nutrition Research Division, the Agricultural Engineering Research Division, and the Market Quality Research Division.

## PROGRAM OF STATE EXPERIMENT STATIONS

Poultry and Eggs. Research on egg quality includes work on the causes and prevention of blood and meat spots and undesirable yolk coloration. Egg shell quality must be good in order to maintain high interior quality in market channels, and the effects of nutrition, heredity, and environment on egg shells are being investigated. The effects of different egg washing techniques on interior quality are also being evaluated. Research is underway on the effect of nutrition and management on chicken and turkey carcass quality traits such as skin pigmentation and a desirable amount of fat.

The total State scientific effort devoted to production influences on animal products research is 45.0 professional man-years.

## PROGRESS - USDA AND COOPERATIVE PROGRAMS

Poultry and Eggs

In a study of eggshell weight, different methods of reducing variability were considered. It was observed that the variability, as measured by the variance and the correlation coefficient was reduced by calculating the ratio between the part and the whole less the part. This was especially noticeable with the albumen, a major component of the whole. (AH e4-10)

## PUBLICATIONS - USDA AND COOPERATING PROGRAMS

None.



INFECTIOUS AND NON-INFECTIOUS DISEASES OF POULTRY  
Animal Disease and Parasite Research Division, ARS

Problem. Annual losses from infectious and non-infectious diseases of poultry, exclusive of parasitisms, are estimated to be at least \$200 million. Continued and expanded basic and applied research are essential to aid in reducing these losses, which inevitably affect cost to the consumer. Added to the initial losses from mortality, reduced weight gains, poor feed utilization, decreased egg production, and lowered quality, are the final losses occasioned by condemnations at dressing plants. United States turkey growers in particular, are faced with a new problem in that a newly discovered infection with a different strain of Mycoplasma is widespread in flocks throughout the country. Resulting condemnation losses at slaughter are often great. The problem is to keep abreast of changing conditions in the field, which present increasingly complex problems requiring basic information.

USDA AND COOPERATIVE PROGRAM

The Department has a long-term program involving biochemists, microbiologists, pathologists, and veterinarians engaged in both basic studies and the application of known principles to the solution of infectious and non-infectious diseases of poultry. Research is being conducted on the diseases at the following locations.

The Federal scientific effort devoted to research in this area totals 17.5 professional man-years. This effort is applied as follows:

Ornithosis 2.7 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with the Universities of California and Minnesota, and the Agricultural Experiment Stations of Oregon and Texas.

Salmonellosis 1.0 at the National Animal Disease Laboratory, Ames, Iowa, and the Southeast Poultry Research Laboratory, Athens, Georgia.

Pasteurellosis 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

Chronic Respiratory Disease Complex 6.3 at the National Animal Disease Laboratory, Ames, Iowa, the Southeast Poultry Research Laboratory, Athens, Georgia, and under cooperative agreements with the Agricultural Experiment Stations of Connecticut, Delaware, Georgia, Massachusetts, New York, North Carolina, Texas, Virginia, and Wisconsin, and with the University of Minnesota.

Newcastle Disease 3.2 at the National Animal Disease Laboratory, Ames, Iowa, the Southeast Poultry Research Laboratory, Athens, Georgia, and under cooperative agreements with the University of Maine and the Wisconsin Agricultural Experiment Station, and under a PL 480 Grant to the Institute for Veterinary Research, Pulawy, Poland.



Leukosis 0.3 under cooperative agreement with the Regional Poultry Research Laboratory, USDA, East Lansing, Michigan.

Infectious Bronchitis 2.0 at the National Animal Disease Laboratory, Ames, Iowa, and the Southeast Poultry Research Laboratory, Athens, Georgia.

#### PROGRAM OF STATE EXPERIMENT STATIONS

Major emphasis is being placed by the State Stations on the respiratory disease complex of poultry (airsacculitis) with particular attention being given to chronic respiratory disease (CRD). Two regional research projects (NE-5 and NC-65) coordinate the work of 20 States and the Department on the respiratory disease complex with the principal effort centered on means for control or eradication of CRD. The role of environment as it affects outbreaks of the disease is being studied and efforts are being made to improve diagnostic materials to locate CRD carriers. Control by means of antibiotic therapy is under investigation and the effectiveness and limitations in using virulent vaccines are being determined by a number of States. Basic investigations are under way to characterize the physical and biological properties of Newcastle Disease, infectious bronchitis, laryngotracheitis and fowl pox viruses.

Other research is concentrated on the important problem of leukosis to determine means of transmission, practical methods for laboratory diagnosis of virus carriers, and effective methods for prevention. Increasing emphasis is being placed on salmonellosis to determine the sources of infection, means of transmission, and practical methods for elimination from poultry products. Basic studies are in progress on the factors affecting resistance of poultry to this disease.

Research also is in progress to provide improved control methods for conditions such as coccidiosis, ascariasis, ornithosis, dissecting aneurysm, Gumboro Disease, mycotic infection, and other important poultry problems.

The States are allotting 93.2 professional man-years to poultry disease research.

#### PROGRESS -- USDA AND COOPERATIVE PROGRAMS

##### A. Ornithosis

At the National Animal Disease Laboratory, Ames, Iowa, basic research is in progress on this problem. It is directed toward relationships between psittacosis-group agents found in wild birds and those found in domestic birds and mammals. In order to understand the relationships between various psittacosis-group agents that affect domestic animals, four strains of these agents were studied to see how they affected laboratory animals, wild and domestic birds, and a domestic mammal. The strains studied were those causing turkey ornithosis, pigeon ornithosis, lamb abortion and lamb polyarthrititis. These strains were inoculated into mice, guinea pigs, chicken



and turkey embryos, pigeons, parakeets, sparrows, turkeys, and sheep. Lethal and infectious endpoints were determined for each strain in each of the species. These determinations revealed specific pathogenicity differences between the strains sufficient to be readily identified in the laboratory on the basis of the effects they cause in just three of the species - mice, guinea pigs, and pigeons.

Of epizootiologic interest were the observations that the virulent turkey strain caused pneumonia in lambs, the pigeon strain produced severe airsacculitis in turkeys and both sheep strains caused disease in turkeys. The significance of these results is that it may be possible for these agents to transfer between hosts, thus possibly to establish intermediate carrier hosts which in turn would perpetuate the disease agents.

(NADL)

(ADP a5-20)

At the University of California, Davis, cooperative work continued. Over 212 chickens of various ages were inoculated by various routes with the C-1 strain of ornithosis agent. No signs were observed in any of the chickens and only two birds presented lesions of pericarditis and plastic exudates over the livers. The virus could be isolated from the blood, saliva, cloaca, liver, and lung for various periods of time beginning 72 hours after exposure. The virus was isolated from the lung and saliva of asymptomatic chickens after a period of 7 weeks, the longest period tested. Complement-fixing antibodies were dependent upon the amount of virus in the inoculum. It was concluded that chickens are resistant hosts but potentially can be carriers of the infection. Stress, such as exposure to CO<sub>2</sub> atmospheres following inoculation, had some influence in the course of infection.

Biochemical analysis of the CF fraction of the antigen prepared from the Herellea-like bacterium showed it to be extremely stable to temperature, pH, and could be salted out with ammonium sulphate. The fraction was demonstrated by chromatography to have a peak at 260 mu.

(California)

(ADP a5-20)

In cooperative studies at the University of Minnesota, St. Paul, routine serologic surveillance, involving some 4,291 samples from 119 turkey flocks in Minnesota and Wisconsin, indicates that about 20% of the flocks tested for ornithosis antibodies gave significant reactions. Individual turkey samples submitted from Iowa, not considered flocks, yielded reactions regarded as suspicious.

A small number of samples obtained from pigeons, chickens, and geese, were considered negative. Sera obtained from pheasants (195 samples) revealed some reactions in the suspect class. Virus isolation attempts from natural cases have been unsuccessful during the year.

A pilot study utilizing bovine sera in the complement fixation test with ornithosis virus antigens indicates a high percentage of reactions at low serum dilutions. The significance of these reactions is unknown.



A synthetic medium has been developed for propagating the Herellea-like bacterium, components of which cross-react with the ornithosis virus.  
 (Minnesota) (ADP a5-20)

At the Texas Agricultural Experiment Station, College Station, histopathologic lesions of meningitis or meningoencephalitis, occurred in 5 of 20 turkeys infected by exposure to sheep experimentally infected with virulent ornithosis virus. This is the first report of central nervous system lesions from this disease in turkeys.

Antigen production was shifted to cell culture methods to give a more specific antigen than the older yolk sac method.

A new highly specific agglutination procedure was developed for use with turkey serums. This procedure is much more rapidly run without elaborate laboratory equipment.

The Jo strain of ornithosis virus was used to experimentally infect chickens. The chickens demonstrated clinical signs up to 3 weeks, but not after. A Psittacosis - LGV group virus was isolated from, and studied in, lambs. Serological evidences indicate this agent to be widespread in lambs in Texas.  
 (Texas) (ADP a5-20)

#### B. Chronic Respiratory Disease Complex

At the National Animal Disease Laboratory, Ames, Iowa, the following work was accomplished:

In outbreaks of Mycoplasma gallisepticum infection in poultry, variations in severity have been observed, as have variations in the incidence of the forms, such as sinusitis or airsacculitis, which occur. These variations may be the result of within-species variation of the causative organism. The following study describes the effects of a particular isolate of M. gallisepticum (strain 1010) on turkeys and compares the effects of intrasinus exposure of this isolate with another.

The serologic, pathologic, and symptomatic responses of adult male Beltsville White turkeys exposed by intratracheal inoculation, intrasinus inoculation, intranasal inoculation, and by contact with inoculated birds to M. gallisepticum isolate 1010 were compared. Signs of infection varied among the groups with tracheitis occurring only in those exposed intratracheally or by contact, and sinusitis occurring only in those exposed via the sinuses. Air-sac lesions did not differ appreciably among the groups in severity or incidence.

Serologic response was initially higher in turkeys exposed by intratracheal or intrasinus inoculation. The hemagglutination-inhibition test was found to be more sensitive than the tube agglutination test for detecting antibodies against M. gallisepticum.



Comparison of the effects of intrasinus exposure of turkeys with M. gallisepticum isolate 1010 and M. gallisepticum isolate 1150, revealed isolate differences. Isolate 1010 caused a slightly higher incidence of sinusitis, but a much lower incidence of tracheitis than isolate 1150. Air-sac lesions did not differ in incidence or severity. The differences observed indicate differences in tissue predilection of the isolates.

(NADL)

(ADP a5-21)

At the Southeast Poultry Research Laboratory, Athens, Georgia, a detailed study was conducted on birds exposed intranasally to Mycoplasma gallisepticum (R strain) under field conditions, at 38 days of age. Groups of exposed birds were transferred from the field at 23 weeks of age and placed in separate laboratory isolations. Subsequent isolation, contact exposure, challenge and serological tests demonstrated that 1) vaccinated birds were shedding Mycoplasma from 29-43 weeks of age, as evidenced by tracheal isolations and spread to susceptible contact birds; 2) vaccinated birds were susceptible to homologous and heterologous challenges at 28 weeks of age, and 3) progeny of exposed birds were not free of Mycoplasma, since isolations were made from day-old chicks and 18-day embryonated eggs.

(SEPRL)

(ADP a5-17)

A method for culturing embryonic chick lung cells was developed and infectious bronchitis virus strain 42 has been adapted to these cells. This permits the study in vitro of infectious bronchitis virus (IBV) in epithelial cells of respiratory tract origin. This virus propagated in these cells is of high titer, is cytopathogenic, and will produce plaques. Another strain of IBV(317), has been carried through 10 serial passages in these cells. This represents the highest passage level in tissue culture of a field strain of IBV from which virus has been recovered. The results support the belief that field strains can be adapted to tissue culture system, and that this system can be used as a tool to investigate the virus.

Growth curves are being constructed in order to determine if there are any differences in the growth characteristics of the various IBV strains. The results of 4 such curves show that the egg adapted strain reaches its peak titer about 5 hours prior to the field strains. The curves produced by 3 field strains are identical.

A procedure for utilizing a serum neutralization test was developed. This procedure, in which a constant virus dose is run against varying dilutions of serum, is expected to be more sensitive in showing antigenic differences in the virus. During the course of these investigations, the virus was found to be unstable at room temperatures. A dilution medium was developed to overcome this difficulty since it is imperative that virus stability be maintained.

(SEPRL)

(ADP a5-23)

In cooperation with the Animal Husbandry Research Division, work was initiated to establish flocks resistant and susceptible to Newcastle disease virus (NDV). Athens Randombred stock has been used in this work. A mean



flock titer for NDV was first determined. Titers were then determined in eggs and chicks from individual hens. Correlations run on data from these trials were not high (r.2). However, a beginning selection has been made, based on families which were most consistently represented in either the high group or the low group. (SEPRL) (ADP a5-18)

At the Connecticut Agricultural Experiment Station, Storrs, under a cooperative agreement with the USDA, researchers ran experiments to compare the performance of birds vaccinated at 8-12 weeks of age with live pathogenic Mycoplasma gallisepticum (MG) with nonvaccinated birds which acquired Chronic Respiratory Disease (CRD) naturally. Results showed that vaccinated birds performed much better in regard to egg production and mortality.

In areas where CRD is endemic, vaccination of flocks, particularly those used as a source of market eggs, appears to be highly desirable until such time that an eradication program can be implemented.

Birds vaccinated at 8-10 weeks of age and examined 18 months later showed high titers to MG. They were also resistant to challenge.

Progeny from vaccinated dams were serologically tested for MG at 8 weeks of age. Of 229 groups of birds (30 birds per group), four groups were positive and 225 were negative. The low rate of transmission suggests that vaccination and subsequent periodic testing of progeny flocks to weed out the positives can be an effective means to eventual eradication of MG in poultry.

A completely synthetic medium was developed for Mycoplasma laidlawii B. This is the first successful attempt to grow any Mycoplasma in a chemically synthetic medium. (Connecticut) (ADP a5-17)

At the Delaware Agricultural Experiment Station, Newark, cooperative research has been directed toward the formulation and operation of an area plan for the control of Chronic Respiratory Disease (CRD). The two States of Delaware and Maryland, as well as the State of Virginia to a lesser extent, are involved. The State Boards of Agriculture are cooperating and the poultry industry association (Delmarva Poultry Industry, Inc.), is giving enthusiastic support. The program is one of controlled exposure using an attenuated broth culture as the vaccine at 8-10 weeks of age on infected replacement flocks. Prevacination blood titers and a series of postvaccination titers are used as a partial criteria of the program's success. In addition the health of the broiler chicks produced, their weight, feed conversion, condemnation rate, cost of producing, and other factors are being included in the evaluation. Also, the disease history of the vaccinated birds and the hatchability of their eggs are other items included in the test. By the summer of 1966, the results of the tests should indicate its feasibility. (Delaware) (ADP a5-17)



In cooperative work at the Georgia Agricultural Experiment Station, Athens, preliminary studies show that it may be possible to increase the resistance of young chickens to Mycoplasma gallisepticum infection by intramuscular injection of living organisms. There are, however, some factors which need to be affirmed before such a procedure could be considered practical. Furthermore, some serological test more sensitive than the agglutination test needs to be developed to determine the immune response in chickens.

(Georgia)

(ADP a5-17)

At the Massachusetts Agricultural Experiment Station, Amherst, cooperative studies gave the following results:

Transmission of Chronic Respiratory Disease (CRD) through cohabitation of CRD serologically positive birds and susceptible birds may occur under certain conditions after a prolonged exposure period. CRD serologically positive birds may be true carriers and may remain carriers for an extended period after the initial disease outbreak. Under certain conditions, shedding may occur which may result in transmission to susceptible contacts. However, in some instances no transmission is apparent, even after prolonged exposure periods. These naturally infected serologically positive birds may yield M. gallisepticum that is capable of producing the disease in artificially inoculated birds and embryos up to 24 months after the disease outbreak.

Birds were experimentally injected with M. gallisepticum. Antibody titers persisted in all but one of the birds after inoculation. The rapid-serum-plate, tube agglutination, and hemagglutination-inhibition tests were in general agreement. Doubtful, unexplained tube agglutination and rapid-serum-plate reactions occasionally occurred in the control groups. The nonspecific hemagglutination-inhibition titers obtained in the control group when the antigen prepared by USDA procedures was titrated against untreated serum samples, could be removed completely when the serum samples were treated with sodium meta-periodate and receptor-destroying enzyme of Vibrio cholerae. This nonspecific reaction did not occur with live broth antigens. M. gallisepticum was reisolated from the respiratory tract up to 5 weeks post-inoculation. After this period, isolation was sporadic with recovery of the organism on the 10th, 11th, 14th, and 19th weeks. This work is still in progress.

Varying degrees of agglutination of M. gallisepticum S6 antigen may be detected in sera from day-old chicks produced by positive dams. Parental agglutinins may be detected in progeny of exposed dams within a short time after the development of CRD agglutinins in the adult birds.

Birds that have undergone a natural outbreak of CRD may exhibit a wide variation in serologic titers to the tube agglutination and hemagglutination-inhibition test procedures. The rapid-serum-plate test gave the most consistent positive results. Samples collected from known negative birds show close agreement by the rapid-serum-plate, tube agglutination, and hemagglutination-inhibition tests.



Attempts to eradicate CRD from two breeding flocks through management and tylosin medication of the dams and progeny have yielded encouraging and promising results.

CRD-free stock can be produced, maintained, and reproduced if adequate sanitation and preventive practices and reliable testing methods are employed. The majority of negative premises continue to remain negative on successive years. The above statements are based on 9 years of observations.

(Massachusetts)

(ADP a5-17)

In cooperative research work at the University of Minnesota, St. Paul, egg dipping and water medication with tylan were used to produce a Mycoplasma gallisepticum-free flock of chickens. This flock was blood tested at 5, 10, 22, 33, and 48 weeks and remained serologically negative to the serum plate and hemagglutination-inhibition (HI) tests for M. gallisepticum.

Two small flocks of "Mycoplasma Free" turkeys are being maintained at the Rosemount Experiment Station. Progeny of one of these flocks is in its third season and the other in its second. Poults from these flocks have remained free of any cultivatable Mycoplasma and are free of the day-old airsacculitis associated with these organisms.

The factors affecting the efficiency of tylan absorption into turkey eggs were studied. Time, temperature, concentration of tylan and the effect of surface reducing agents were studied. A time of 15 minutes with a 35°F temperature differential between the dip solution and the eggs, and 3,000 ppm have been selected as the most practical for field use. Surface reducing agents have little effect at this concentration.

A salvage program for two valuable turkey breeding flocks infected with Mycoplasma gallisepticum is being tried. This program consists of treating the infected flock (both hens and toms). The eggs from these flocks are dipped in tylan. The poults are being water medicated for 5 days at one day of age. The poults from these flocks are inspected periodically for signs of respiratory disease and tests are conducted for M. gallisepticum antibodies. The egg transmission of the "N" strain of avian Mycoplasma was studied. Two experiments were conducted in an effort to determine the nature of the egg transmission of this strain. The rate of transmission appears to increase as the production period increases. The adult hen is able to localize the infection in the upper respiratory tract. Consequently no Mycoplasma could be isolated from the air sacs of artificially infected hens by the aerosol inhalation method. Further studies on the association of the length of time in production with the transmission rate are being conducted.

(Minnesota)

(ADP a5-17)

Field investigations were conducted on thirty-one clinical outbreaks of infectious sinusitis in turkeys during the past year. The major reason for the outbreaks was egg transmission from an infected breeder flock (59%). The next important source was exposure from infected chickens (15%). Imported hatching eggs and lateral transmission also were important causes of outbreaks.



Studies were continued on the effect of environmental conditions on the airsacculitis syndrome in turkeys. Three experiments were conducted in an effort to raise "Mycoplasma Free" fryer-roaster turkey flocks. The eggs were dipped in varying concentrations of tylosin and in one experiment the day-old poults were water medicated. The airsacculitis observed at the processing plants was very minimal in all three flocks. However, a Mycoplasma free status was not attained.

Air samples were taken in the environmental turkey buildings throughout the last two experiments. The counts of bacteria per cubic foot of air increase very sharply the first four weeks. They were found to be somewhat higher during the winter months. This may result from a reduced air flow as a result of the low ambient temperatures.

The voluntary control program for Mycoplasma gallisepticum which consists of a 100% testing by all turkey breeders continues to be effective in minimizing this infection in Minnesota turkeys. Only 105 birds of 659,928 tested were submitted to the University for further laboratory analysis.

A survey of the incidence of the "H" serotype of Mycoplasma in Minnesota turkeys was conducted. Sixty-four percent of all samples tested reacted to the serum plate test antigen developed at the University of Minnesota.  
(Minnesota) (ADP a5-21)

In cooperative studies at the New York Agricultural Experiment Station, Ithaca, researchers found that the failure of a live pathogenic culture of Mycoplasma gallisepticum to induce 100% protection against egg transmission indicated that this method of immunization into the air sac by itself cannot be used to initiate production of disease-free progeny. Vaccination by intranasal instillation with live cultures likewise did not afford complete protection after challenge. The vaccination itself induced egg transmission in some instances.

An attempt to produce clean progeny from infected breeding stock by dipping the eggs in Tylosin solution has given encouraging results thus far. Special care must be taken to avoid adventitious infection during the growing and laying period on the farm. No evidence of infection has been found in 9 dipped hatches. The undipped eggs from 3 of these hatches yield infection.

Under experimental conditions, brief (4 days) contact exposure to infected birds or exposure to contaminated premises for 15 weeks failed to induce M. gallisepticum infection in clean chickens.

A simple medium incorporating coagulated egg yolk has been found highly effective for growing most avian mycoplasma. (New York) (ADP a5-17)



In cooperative research at the North Carolina Agricultural Experiment Station, Raleigh, extensive field experimentation has been undertaken in an evaluation of experimental planned infection (EPI) in the immunization of chickens against Mycoplasma gallisepticum, employing live culture inocula. To date individual inoculations have been made on slightly more than 900,000 broiler-type replacement pullets between 8 and 18 weeks of age via the intranasal or posterior thoracic air sac routes. One-tenth ml. of broth culture of the California Chick-F isolate has been used on all flocks. Approximately 1% of the birds in candidate flocks are tested (serum-plate) as a means of serologically classifying flocks prior to inoculation.

Clinical symptoms following EPI inoculations have been relatively insignificant. However, some flocks have required medication. Several flocks are now under investigation to determine the validity of suspected concurrent virus respiratory infections. Two flocks subjected to laryngotracheitis vaccination approximately 2 weeks post EPI, developed typical air-sac disease symptoms and lesions and required medication. Progeny data from EPI flocks is not sufficient at this stage of the experimentation to justify application.

Two hundred and fifty one flocks have been serologically tested at least once for M. gallisepticum antibodies, of which 82% were positive as a result of natural exposure. Many serologically positive flocks revealed that the rate of advancement to a 100% positive status was slow and the degree of agglutination reaction was only moderate. Twenty M. gallisepticum-clean parent and grandparent flocks have been negative on all tests to date. However, some of these flocks are not yet sexually mature.

Cross agglutination between M. gallisepticum antigen and M. synoviae antibodies is an important consideration in flock classification. It is essential that M. gallisepticum-clean flocks yielding weak positive reactors be tested for M. synoviae before the clean status is revoked. The need for a suitable M. synoviae antigen becomes obvious.

(North Carolina) (ADP a5-17)

At the Texas Agricultural Experiment Station, College Station, in cooperative studies on infectious sinusitis eradication, 227 breeder flocks, representing 218,949 turkeys, participated in the 1964 M. gallisepticum eradication program. Five M. gallisepticum-infected flocks were identified and marketed.

In work with M. gallisepticum antigens, cooperative studies with the Animal Disease Eradication Division of the USDA were continued and standard production and testing protocols for M. gallisepticum plate, tube and hemagglutination-inhibition (HI) antigens were developed. These findings were made available to industry and at least one commercial firm is now producing antigen.

Condemnation studies revealed that 55.5 percent of turkey condemnation in Texas are due to airsacculitis. However, M. gallisepticum infection was responsible for only 83 of 633 birds condemned in this category. This



confirms the value of the infectious sinusitis eradication program. However, it does point out the need for additional research on causes of airsacculitis, particularly fowl cholera, aspergillosis, and other undetermined causes.

Quail bronchitis virus was shown to have little potential as a "triggering mechanism" for air sac syndrome. However, evidence was obtained that some strains might be more pathogenic to broilers than heretofore supposed.

(Texas)

(ADP a5-17)

Cooperative work at the Virginia Agricultural Experiment Station, Blacksburg, has shown that a disease of chickens characterized by reduced weight gain and retained caseous yolk sacs was reproduced by dipping incubating eggs in a suspension of 0103 serotype Escherichia coli obtained from a field flock affected with a similar condition.

Social stress was produced in chickens between 56 and 70 days of age by moving male White Leghorn chickens into cages with other birds according to a schedule which kept contact with previously encountered birds to a minimum. At the end of the 2-week social stress period, the stressed birds were more resistant to pathogenic strains of Escherichia coli inoculated via the air sac than the unstressed controls. Social stress did not increase resistance to birds similarly inoculated with Mycoplasma gallisepticum.

(Virginia)

(ADP a5-17)

In cooperative research studies at the Wisconsin Agricultural Experiment Station, Madison, chickens exposed artificially to poultry house dust in an environmental chamber showed no gross or microscopic signs of damage to the respiratory tract after 6 hours of continuous exposure, or 2-hour exposures for 8 consecutive days. Chickens exposed "naturally" to ammonia carbon dioxide, and dust in the environment of a poultry house for 6 days, showed some loss of cilia from the epithelium of the upper portion of the trachea and the turbinates and an increase in mucus secreting goblet cells. Dust particles were present in the macrophages of the lungs. When these chickens were exposed to a secondary stress in the form of a respiratory infection initiated by an aerosol of Newcastle Disease virus, there was, as compared to control birds receiving only the NDV aerosol, a possible shortening of the mean death time and possible increased percent mortality.

Attempts have been made to develop a serological test which will overcome some of the disadvantages of the conventional colony inhibition test used for serological classification of the mycoplasma. A test in which porous paper strips are used as a vehicle for interaction between the unknown strains and known antisera has shown some advantages and some disadvantages. Some strains of the organism do not give good reactions in early culture passages.

(Wisconsin)

(ADP a5-21)



### C. Salmonellosis

At the Southeast Poultry Research Laboratory, Athens, Georgia, studies have been completed on the flagellar antigenic balance and hemagglutination properties of 565 cultures of Salmonella typhimurium isolated from avian sources in the United States. Approximately 95% of these cultures have been demonstrated to produce hemagglutination of avian red blood cells. Based on naturally occurring high levels of phase 1 and phase 2 flagellar antigens, 33 cultures of S. typhimurium have been selected for further antigen preparation and growth studies. These cultures are being subjected to detailed single-factor somatic antigenic analysis in further characterizing their sensitivity and antigenic balance for use in detecting serological reactors to S. typhimurium agglutination tests.

Techniques have been developed and are presently under further detailed study for sampling microbial penetration through the shell and shell membranes of chicken eggs under varying conditions of temperature and humidity. Salmonella typhimurium is being used as the test organism. These methods involve the mechanical separation of the eggshell and its membranes prior to exposing selected areas of the shell surface to known numbers of bacterial cells. These studies are being extended to evaluate the effects of egg treatments such as formaldehyde fumigation and egg washing on the penetration patterns of Salmonella organisms. Preliminary studies have indicated that the penetration rate of these bacteria through the shell surface is much more rapid than previously accepted and described using less refined methods of sampling.

(SEPRL)

(ADP a5-2(Rev.))

### D. Pasteurellosis

At the National Animal Disease Laboratory, Ames, Iowa, in studying the host-parasite relation of fowl cholera, experiments were designed to study the action of the organism and the reaction of the host. The most pronounced and significant lesion of acute fowl cholera has been found to be generalized passive hyperemia which resulted from cardiac insufficiency, atony of veins and capillaries. These lesions were indicative of the syndrome of shock which is often attributed to the action of endotoxins.

During the past year studies were made of the structure and characteristics of P. multocida as related to its pathogenesis. Particulate antigens were isolated from noncapsulate avirulent cells of two immunogenically distinct strains (X-73 and P-1059) of P. multocida. They possessed many of the properties of endotoxins. Almost 100% of chickens given sublethal injections of these antigens were protected when challenged with live organisms which killed 100% of the controls. The same degree of protection was obtained in mice. Injection of various amounts of the antigens into mice, rabbits, or chickens produced moderate to severe toxic effects such as depression and diarrhea. Death frequently followed. Chickens had the same signs after injection as has been observed with cases of acute fowl cholera.



It is concluded that in the pathogenesis of fowl cholera, the ability of P. multocida to form a capsule is significant. The capsule allows the organism to grow and produce in the host an endotoxin which is responsible for the signs observed in acute fowl cholera. A vaccine prepared with the endotoxin will stimulate immunity against the natural disease.

The immune response stimulated by each strain of P. multocida used in fowl cholera vaccines can be determined by challenging groups of vaccinated birds. It would be advantageous to have a serologic test that would indicate the immune response in birds stimulated by these organisms and differentiate immunogenic strains. Experiments were designed to compare the immune response of chickens and turkeys to 2 known and 2 unidentified immunogenic strains, and to compare the passive-immunity test, agar double-diffusion test and serum-plate agglutination test to the immune responses.

The immune status of chickens and turkeys to P. multocida could not be determined by these 3 tests. Immune and hyperimmune chicken serum did not induce passive immunity in mice. Strains of P. multocida that differed in their fermentation, agglutination, immunogenic, and pathogenic characteristics could not be differentiated on the basis of the agar double-diffusion test. The agglutination test did not clearly indicate the immune status of chickens and turkeys that were vaccinated, tested serologically and exposed with homologous cultures. There was very little cross-agglutination with some cultures, but there was cross-immunity. Vaccines containing only one strain of P. multocida gave better immunity in turkeys to homologous challenge than bivalent or trivalent vaccines. However, all vaccines gave good protection in chickens, with the exception of those challenged intravenously. Turkeys were more susceptible to P. multocida than chickens, and mature chickens were more susceptible than young chickens.

It is apparent as a result of these studies that there is no complete correlation of the serologic, biochemic, immunogenic, or pathogenic methods of typing P. multocida and that these serologic tests cannot determine the immune status of chickens and turkeys.

Two strains of Pasteurella multocida (X-73 and P-1059) of avian origin are commonly used in fowl cholera vaccines in the United States. Heddleston reported that these strains differed in their immunogenic, serologic, pathogenic and biochemical properties. Namioka and Musata typed these two strains on the basis of capsular and somatic antigens and placed X-73 in serotype 5:A and P-1059 in serotype 8a:A. They also described a serotype 9:A which was isolated from a turkey in the United States, and a serotype 8:A which was very similar to 8a:A. They reported that serotypes 5:A, 8:A and 9:A did not cross-immunize. Since only 2 immunogenic types are included in fowl cholera vaccines in the United States, a comparative study of serotypes 9:A, 8a:A and 8:A was undertaken.



The serotypes could not be differentiated on the basis of immune response. Each isolate gave a high degree of immunity against homologous and heterologous challenge. On the basis of the tube-agglutination test and cross-absorption of immune chicken serums, serotypes 8a:A and 9:A were similar. Serotype 8:A contained an additional antigen (positive at 1:20) which was not detected with the serum plate test. Biochemical fermentations were similar with the exception that one strain did not ferment xylose. For the present, at least, only two serotypes of Namioka and Murata, 5:A (X-73) and 9:A (P-1059) are necessary in the production of fowl cholera stock vaccines in the United States. (NADL) (ADP a7-25)

#### E. Newcastle Disease

At the National Animal Disease Laboratory, Ames, Iowa, basic research on the Newcastle Disease problem is directed toward improving inactivated vaccine for susceptible chickens at various ages. (NADL) (ADP a5-18)

Cooperative research at the University of Maine, Orono, has been directed toward the prevention of Newcastle disease by the use of killed vaccine. The vaccine has proved very effective for control of the disease.

A Specific Pathogen Free (SPF) program has been conducted on broiler and breeder flocks during the past year. A rigid set of standards for isolation and husbandry are required to conform to the program. For the entire period of study at least 700,000 tests have been made on breeding hen flocks in an effort to eradicate  $S_6$  PPLO. (Maine) (ADP a5-18)

Cooperative research at the Wisconsin Agricultural Experiment Station, Madison, was in four areas - 1) accession and identification of new isolates; 2) study of virus genetics; 3) study of pathogenesis, and 4) study of epizootiology.

In the first area, 14 cultures of Newcastle Disease virus (NDV) were received in the past year. Most of them brought important questions. The virulent culture recovered from a chicken in Maine resembled the virus used in producing the killed vaccine which the flock had received. The question was, were the two cultures the same? While identity can never be conclusively established, the plaque morphology, physical stability, biological properties in embryos and chickens were such that the culture could not be distinguished from the vaccine stock. The supplier suggested that the vaccinated flock was probably contaminated by an unknown contact with the nearby farm where the vaccine potency tests were conducted rather than by receiving un-inactivated virus in the vaccine.

Nine isolates that had been recovered from vaccinated chickens were supplied from Quebec. All these cultures contained Newcastle disease virus. The cultures differed in plaque morphology, embryo and chicken pathogenicity, and probably represent at least 3 strains. None of the cultures can be classed as exotic as enteric lesions are not produced in chickens.



A culture of Newcastle disease virus with very low pathogenicity for chicken embryos was recovered from a laryngotracheitis virus isolate obtained near Bakersfield, California. It was first believed to be Yucaipa virus. Two cultures have not yet been examined. One is an exotic strain isolated from Indonesian Cockatoos. The other is a strain of Newcastle disease virus isolated from a bronchitis vaccine that is being marketed in the United States.

In the genetics area, isolates of Newcastle disease virus on initial recovery and after long term propagation in laboratory host systems consist of heterogeneous populations, the components of which differ in plaque type and in many other identifiable characters. The segregated components can be maintained pure for plaque type for only a limited number of passages. The relationship of plaque type to other characters, the degree of phenotypic variation, and the rate of mutation are subject of study.

A system for establishing degrees of serological relationship based on an index which uses kinetics of neutralization data appears feasible.

Studies in the third area revealed that the pathogenesis of Newcastle disease virus in chickens, following an aerosol exposure, is affected by environmental conditions (air pollutants) and by the immune status of the individual. Only aerosols of a size that penetrate the entire respiratory system appear to induce solid immunity.

In the area of epizootiology, neutralizing (titers of 1,000 to 100,000) and hemagglutinin-inhibiting (titers of 40-320) substances for Newcastle disease virus were found in a high percentage of both the juvenile and adult members of a migratory bird population. Adults will die if massive doses of virus are given. Lesser doses that would be lethal to chickens are tolerated and the birds develop neutralizing antibody. Susceptibility of juveniles and persistence of antibodies in adults and juveniles is under investigation. On the basis of preliminary data, it appears that the lethality gradient of Newcastle disease virus strains for chickens is not true for this host. The relationship of Newcastle disease virus and this migratory bird will be carefully studied as the perpetuation of the infection in them is most readily understandable if one assumes a cycle of disease independent of the domestic fowl.

(Wisconsin)

(ADP a5-18)

#### F. Infectious Bronchitis

At the National Animal Disease Laboratory, Ames, Iowa, basic studies on the causative agent of infectious bronchitis are being conducted. A comparison of the embryonating egg, chicken embryo kidney (CEK), and chicken embryo liver (CELi) cell cultures revealed the egg to be 12-40 times more sensitive to infectious bronchitis virus (IBV) than CEK cells and about 500-1000 times more sensitive than CELi cells. Plaque counts in both CEK and CELi cells followed a linear relationship with virus concentration, indicating that one virus particle initiates a plaque. Fifty percent cell culture infective



dose titers in kidney and liver cell cultures were equivalent to plaque titers showing that all cell culture infecting virus was also plaque forming virus. There were no differences in adsorption of virus to both cell types. One step growth curves in liver cells show that the eclipse phase was longer and virus production and release was much slower than in kidney cells. Virus particles could not be selected nor altered by adaptation to increase the plaquing efficiency on the different cell types.

(NADL)

(ADP a5-23)

#### G. Avian Encephalomyelitis

At the National Animal Disease Laboratory, Ames, Iowa, recent field studies of avian encephalomyelitis have provoked the question of marginal vitamin E deficiency as a factor in the development of this viral disease. The studies described herein are an effort to determine this possibility.

Preliminary experiments, using semisynthetic vitamin E deficient diets, were conducted to determine the type and severity of lesions as well as the time of their occurrence. Syndromes of encephalomalacia, exudative diathesis, and muscular dystrophy were produced in day-old chicks placed on a torula yeast base diet. Changes in the per cent of lipid were responsible for these variations in the disease process. Nervous system lesions occurred only in chicks on 9 per cent dietary lipid. The muscle lesions were shown to be due to direct damage to mitochondria.

The dietary regimen producing neural lesions was to investigate the effects of avian encephalomyelitis virus in vitamin E deficient chicks. It was shown that vitamin E deficient chicks do not develop as severe an infection as non-deficient chicks. It is doubtful, therefore, if vitamin E deficiency would be a predisposing factor in the development of avian encephalomyelitis.

(NADL)

(ADP a5-27)

#### PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

##### Ornithosis

Bates, H. A., Pomeroy, B. S., and Reynolds, D. P. 1965. Ornithosis: Immunization of Turkeys with Miyagawanella of low virulence. Avian Diseases 9:220-226.

Bates, H. A., Pomeroy, B. S., Seal, U. S., and Jay, A. R. 1965. Ornithosis: Experimental Immunofluorescent Studies. Avian Diseases 9:24-30.

Beasley, J. N., and Walkins, J. R. 1965. Ornithosis in Chickens: Experimental Infection with a Virulent Turkey Strain of the Agent. Southwestern Vet., 18:2:199-202.

Kinjo, T., and Bankowski, R. A. 1965. Properties of Complement-fixation antigens prepared from the 6B6 ornithosis agent and Herellea-like (HL) bacteria with immune serums. Avian Diseases 9:3:359-367.



Livingston, C. W., Jr., Moore, R. W., Redmond, H. E., and Hardy, W. T. 1965. Polyarthrititis Virus - A Cause of "Stiff Lambs" in Texas. Southwestern Vet., 18:4:279-281.

Pierce, K. R., and Moore, R. W. 1965. Meningoencephalitis in Turkeys Experimentally Infected with Ornithosis. Avian Diseases 9:2:266-272.

#### Chronic Respiratory Disease Complex

Anderson, D. P., Cherms, F. L., and Hanson, R. P. 1964. Studies on measuring the environment of turkeys raised in confinement. Poult. Sci., 43:305-318.

Anderson, D. P., Beard, C. W., and Hanson, R. P. 1964. The adverse effects of ammonia on chickens including resistance to infection with Newcastle disease virus. Avian Diseases 8:369-379.

Anderson, D. P., and Hanson, R. P. 1964. The influence of the environment on viral diseases of poultry. Presented at the 101st Ann. Meet. AVMA, Chicago, July 19-23. Avian Diseases 9:171-182.

Chute, H. L., Stauffer, D. R., and O'Meara, D. C. 1964. The Production of Specific Pathogen Free Broilers in Maine. Agr. Exp. Sta. Bull. 633.

Chute, H. L., Stauffer, D. R., Cuozzo, R. F., and O'Meara, D. C. 1964. Progress in PPLO eradication in chickens in Maine. Maine Farm Research July.

Chute, H. L., Cuozzo, R., Stauffer, D. R., and MacDonald, Vaughn. 1965. The Commercial Production of PPLO Free Chickens. Can. Vet. Jour., Jan. pp. 16-21.

Chute, H. L. 1964. Studies on the Commercial Production of SPF Poultry. Pres. USLSA Meet. Memphis, Tenn., Oct. 17-21.

Domermuth, C. H., Nielson, M., Freundt, E. A., and Birch-Anderson, A. 1964. Ultrastructure of Mycoplasma species. Jour. of Bact., 88:727-744.

Domermuth, C. H., Nielson, M., Freundt, E. A., and Birch-Anderson, A. 1964. Gross morphology and ultrastructure of Mycoplasma gallisepticum. Jour. of Bact., 88:1428-1432.

Dutta, S. K., Dierks, R. E., and Pomeroy, B. S. 1965. Electron microscopic studies of the morphology and stages of development of Mycoplasma gallisepticum. Avian Diseases 9:241-251.

Fabricant, J., and Boyer, C. I. Jr. 1964. Mycoplasma other than M. gallisepticum associated with airsacculitis in turkeys. Proc. 36th N.E. Conf. on Avian Diseases, North Carolina State University, Raleigh. June.

- Gross, W. B. 1964. Retained caseous yolk sacs caused by Escherichia coli. Avian Diseases 8:438-441.
- Hall, C. F., Flowers, A. I., Grumbles, L. C., and Moore, R. W. 1965. Turkey Condemnation Causes. Technical Report No. 1, Texas Agri. Exp. Sta.
- Olesiuk, O. M., Van Roekel, H., and Chandiramani, N. K. 1965. Control of experimental Mycoplasma gallisepticum infection in young chickens with tylosin and other antibiotics. Avian Diseases 9:67-77.
- Patterson, W. C. 1965. Recent Research on the Chronic Respiratory Disease Complex. Presented at Dedication of South Central Poultry Research Laboratory, State College, Mississippi, May 28.
- Patterson, W. C. 1965. Environmental Research Related to Poultry Health Problems. Presented at 58th Ann. Meet. American Soc. of Agricultural Engineers, Athens, Georgia, June 22.
- Payne, J. B. 1965. The Role of Quail Bronchitis Virus as a Possible Precipitating Factor in "Air Sac Syndrome" of Chickens. Thesis, Texas A&M University.
- Sabry, Mohy, and Fabricant, Julius. 1965. An egg yolk overlay medium for the cultivation of avian mycoplasma. Proc. 37th N. E. Conf. on Avian Diseases, University Park, Pennsylvania, June.
- Vardaman, T. H. 1965. Field Experiences on Mycoplasma gallisepticum-free Chickens. Pres. Georgia Veterinary Medical Assn. Annual Meeting, Jekyll Island, Georgia, June 19.

### Salmonellosis

- Williams, J. E. 1964. Salmonellosis - A Problem Challenging both Understanding and Action. American Poultry and Hatchery Federation News, August-September.
- Williams, J. E. 1964. Paratyphoid and Arizona Control in Poultry. Pres. Poultry Management School, Mississippi State University, State College. September.
- Williams, J. E. 1965. A Technique for Sampling Microbial Penetration Through the Shell and Shell Membranes of Chicken Eggs. Presented Southern Conf. on Avian Diseases, Gainesville, Florida. April.
- Williams, J. E. 1965. Salmonella Control in Poultry Flocks. Pres. Southeastern Poultry and Egg Conference, Atlanta, Georgia. January.
- Williams, J. E. 1965. Paratyphoid Control in Poultry. Presented "Broiler Day," University of Georgia, Athens. May.



Pasteurellosis

- Heddleston, K. L., and Watko, L. P. 1965. Fowl Cholera: Comparison of Serologic and Immunogenic Responses of Chickens and Turkeys. Avian Diseases 9:3:367-376.
- Heddleston, K. L., Rebers, P. A., and Ritchie, A. E. 1965. Immunizing and Toxic Properties of Particulate Antigens from Two Immunogenic Types of Pasteurella multocida of Avian Origin. Federation Proc. 24(2):3149.

Infectious Bronchitis

- Lukert, P. D. 1965. Comparative sensitivities of embryonating chicken's eggs and primary chicken embryo kidney and liver cell cultures to infectious bronchitis virus. Avian Diseases 9:2:308-316.

PARASITES AND PARASITIC DISEASES OF POULTRY  
Animal Disease and Parasite Research Division, ARS

Problem. Parasites and parasitic diseases probably cost the poultry industry many millions of dollars annually by causing intestinal disturbances, emaciation, retarded growth, reduced egg production, and deaths. Parasites are ubiquitous, many times insidious, and often overlooked until birds are damaged irreparably. Early diagnosis is difficult, and reliable treatments for many devastating parasitoses are not available. Moreover, some management practices, intended to avoid spread of parasites and to control them, have been found ineffectual as is shown by the increasing importance of certain parasites in broiler production. The problem is to develop, through a planned, balanced program of basic and applied research, methods for preventing, controlling or eradicating parasitic diseases, thus affording economical production of healthy poultry and sound products in supplies adequate to meet the needs of an expanding population.

USDA AND COOPERATIVE PROGRAM

The Department has a continuous long-term program involving parasitologists, biologists, and chemists, engaged in both basic studies and the application of known principles to the solution of the problem of parasites and parasitic diseases of poultry.

The Federal scientific effort devoted to research in this area totals 4.0 professional man-years. This effort is applied as follows:

Control of Coccidiosis 2.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Biology of Nematode Parasites 1.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Biological investigations of Protozoan Parasites and Parasitic Diseases, with Special Reference to those of the Gastrointestinal Tract 1.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

PROGRAM OF STATE EXPERIMENT STATIONS

The major emphasis in this area is being placed on the problem of coccidiosis. The interaction of nutrition, bacterial organisms and coccidia in bringing about disease outbreaks is being investigated. The effectiveness of coccidial vaccines is under evaluation and factors are being determined which influence the immunity obtained from these vaccines. Micro-environmental conditions favoring development of infective coccidial oocysts are being studied. The problem of coccidiosis in turkeys is under evaluation and the important species involved in outbreaks are being determined.



Several States have research in progress on blackhead to develop improved means for controlling this parasite.

There are 5.8 professional man-years allocated to research on poultry parasites at the States.

#### PROGRESS -- USDA AND COOPERATIVE PROGRAMS

##### A. The Biology of the Nematode Parasites of Poultry and Related Birds with Special Reference to the Application of Findings to Control Measures.

At the Beltsville Parasitological Laboratory recent experimental work confirmed previously reported findings that the drug, thiabendazole, was very effective when used to remove poultry gapeworms from young turkeys. In three experiments, mash containing 0.5 percent of thiabendazole by weight was fed ad libitum for periods of 17, 17, and 20 days beginning on the day of experimental infection and on the 28th and 30th day post-infection, respectively. All of the worms were removed from the 33 treated birds. Twenty untreated controls harbored 247 pairs of gapeworms. Possible systemic action of the drug was indicated by its action against worms that had already reached the trachea before treatment was initiated. No adverse effects attributable to the drug were noted. (BPL) (ADP b4-10)

##### B. Biological investigations of Parasites and Parasitic Diseases of Poultry with Special Reference to those of the Gastrointestinal Tract.

Through research at the Beltsville Parasitological Laboratory, an effective means has been developed for detecting contamination of soil in poultry yards, gamebird runs, and turkey ranges with cecal worms and the protozoan parasite that causes blackhead. Earthworms, preferably the common fishing worm, Lumbricus, collected at appropriate times from such soil, are fed to birds susceptible to the parasites sought, and to blackhead. Evaluations are based on 1) the occurrence of blackhead in birds so fed; 2) the occurrence of cecal worms in them, and 3) the presence of Histomonas, ascertained by post-mortem examinations, by ante-mortem fecal examinations, and clinical observations. From predetermined values based on prior testing, the extent of contamination of the test plots, and the interval since such contamination occurred can be approximated.

Histomonas meleagridis can be attenuated by serial in vitro passage, causing it to lose its virulence and invasive power, but still retain much of its ability to immunize chickens when inoculated into them, rectally. To a lesser extent, this is also true of turkeys. With improved methods, this could have practical applications, at least with some breeds or strains of birds.

Critical studies of stages in the life cycle of one of the poultry coccidia, Eimeria acervulina, in its host, the chicken, uncovered several new facts. There are 3, and possibly 4, generations of merozoites produced in the



endogenous life cycle of Eimeria acervulina. The first two generations develop deep within the glands of Lieberkühn. Their development is exceedingly quick. Mature schizonts of the 2nd generation are present at 48 hours and immature schizonts of the 1st generation are not present before 30 hours. This means that two generations are produced in 18 hours. The 3rd generation develops superficially in the villar epithelium. A 4th generation, morphologically similar to the 3rd, is most probable. Assuming only 3 generations and using suitable equations to calculate the theoretical yield, it was found that the actual oocyst yield from a given dosage of oocysts was really more than the theoretical. Assuming the more logical 4 generations, the actual oocyst yield from the same dosage was 30-40% lower than the theoretical - a level it should be at assuming a substantial number of sporozoites from the dosage are lost in the host.

After emerging from the oocysts in the intestine of the chicken, the sporozoites enter the tips of the intestinal villi and pass into the lamina propria, or core, of the villus. Within the lamina propria, they are engulfed by macrophages and taken to the duodenal glands of Lieberkühn. The macrophages serve as a defense mechanism against infection as well as a transport system for the sporozoites. Most of the sporozoites are either ejected into the gland lumen or destroyed by the macrophages. The number destined to develop to 1st generation schizonts most probably depends on the inherent potential of the oocyst culture from which they come and on the quantity and/or quality of cell stimulus received. No 1st generation schizonts were found after 48 hours. It is possible that the schizogonous cycle, like the alarm on a clock, is "timed" to "go off" when the proper host cell stimulus is provided and the production of 1st generation schizonts stops when the upper threshold of the inherent potential is reached.

(BPL)

(ADP b4-11)

#### PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

##### Biology of the Nematode Parasite of Poultry

Hwang, J. C. 1964. Hemogram of turkey poults experimentally infected with Syngamus trachea. Avian Diseases, 8:380-390.

Wehr, E. E. 1964. Anthelmintic activity of thiabendazole against the gapeworm, Syngamus trachea, in turkeys. J. Parasitol., 50:60.

##### Biological Investigations of Protozoan Parasites

Lund, Everett E. 1964. Biological Control of Animal Parasites. Symposium, Pest Control by Chemical, Biological, Genetic, and Physical Means, Montreal

##### Coccidiosis of Poultry

Doran, David J., and Farr, Marion M. 1965. Susceptibility of 1- and 3-day-old chicks to infection with the coccidium, Eimeria acervulina. J. Protozool. 12(2):160-166.



**POULTRY INSECTS**  
**Entomology Research Division, ARS**

Problem. Numerous species of insects, mites, and ticks are common pests of poultry throughout the country and if not controlled can make poultry raising unprofitable. They cause poultry to look unsightly, reduce weight gains and egg production, and mar the skin, which results in downgrading of quality and lower market prices. Pests such as black flies and mosquitoes transmit leucocytozoon and fowl pox disease which exact a heavy toll in death and unthrifty poultry each year. House flies spread parasites and enteric diseases which may decimate flocks. Safer, more effective non-residue-forming insecticides are needed to combat these poultry pests and vectors of diseases of poultry. Better materials are needed for direct application to poultry or in poultry houses to control lice, mites, and ticks and for use as larvicides or fly baits to control flies. Materials are especially needed which, when given in feed or water, would act systemically to control external pests and render droppings toxic to fly larvae. Exploratory studies are needed to investigate possibilities of developing attractants, chemosterilants, antimetabolites, or other new methods of combatting poultry pests. Biological and sanitation methods of control offer excellent possibilities for control and need to be emphasized. There is a special need to investigate the roles of insects, ticks, and mites in the transmission of poultry diseases.

**USDA AND COOPERATIVE PROGRAM**

A continuing study is underway in the Department involving basic and applied research on insects, mites, and ticks that affect the health and productivity of poultry. Studies are designed to determine breeding habits and reproductive capacities of various poultry pests and to gain further knowledge on the nature of resistance of these pests to certain insecticides. Work at present is devoted mostly to lice and the northern fowl mite, and to the house fly, which breeds abundantly in poultry droppings. A newly expanded program aims to find new ways to control pests of poultry with special emphasis on use of chemosterilants, antimetabolites, attractants, and non-insecticidal materials and methods. Current studies in this field are largely limited to house flies. They include investigations of physical and mechanical methods for controlling house flies being conducted in cooperation with the Agricultural Engineering and Animal Husbandry Research Divisions.

Research is concerned with the development of more effective insecticides for the control of poultry pests. New chemicals are screened in the laboratory for contact and residual toxicity to lice and mites attacking poultry and to house flies, and promising ones are tested for effectiveness under practical field conditions. New methods of utilizing insecticides more efficiently and safely are being investigated, with special attention to finding materials that, when given orally in water or feed, will act systemically to kill lice and mites on the poultry, and render the droppings toxic

to fly larvae. Efforts are also being given to methods of sanitation and management to control breeding in accumulations of manure in poultry houses. Studies are conducted to determine the occurrences of residues in tissues of poultry treated with insecticides. Work is done in cooperation with State Experiment Stations and poultry raisers at Gainesville, Fla., Stoneville, Miss., Corvallis, Oreg., and Kerrville, Tex. Additional research is conducted at Lake Charles, La. Research is conducted at the University of California at Berkeley and the University of Georgia under grant support.

The Federal scientific effort devoted to research in this area totals 2.2 professional man-years. Of this number, 0.4 is devoted to basic biology, physiology, and nutrition; 0.9 to insecticidal and sanitation control; 0.2 to insecticide residue determination; 0.6 to insect sterility, attractants and other new approaches to control; and 0.1 to program leadership.

The Federal support devoted to research in this area under grant support totals 0.4 man-years. Of this number 0.1 is devoted to basic biology, physiology and nutrition and 0.3 to biological control.

#### PROGRAM OF STATE EXPERIMENT STATIONS

The States are performing both basic and applied research on poultry insects. Studies are in progress to determine the distribution and amount of damage caused by the more than 50 external parasites of poultry in the various states. As the extent of injury is determined, harmful species are studied to obtain information on their life histories and ecology. For example, in the northern fowl mite, the rate of population increase, the incubation periods of the eggs and duration of the immature stages are being studied. The effects of various population levels in production and fertility of eggs are also under investigation. The development of laboratory rearing methods is a pre-requisite for much of this research.

Control studies include comparisons of insecticides. New materials are constantly being evaluated as replacements for chemicals to which some poultry pests have become resistant. Treatment methods range from feed additives, which prevent fly development in manure or provide systemic control of parasites on the birds to direct or area applications. Insecticide residue analyses are performed to determine concentrations of parent compounds and metabolic products at specific intervals following applications. Rates of degradation are determined by bioassay and chemical analyses in eggs and tissues of the birds.

Studies are in progress on the effects of insecticides and management practices on the natural enemies of pest flies. Cultural practices which favor the biological control agents are integrated with selective applications of insecticides to minimize or prevent the destruction of natural enemies, but still obtain maximum kills of pest flies.



Research on external parasites of poultry also includes detailed studies to identify vectors of diseases. Suspect vectors are reared and fed on infected birds and transferred to healthy ones. Life history studies are performed on many of these insects.

There are 6.0 professional man-years committed by the States to research on poultry insects.

## PROGRESS--USDA AND COOPERATIVE PROGRAM

### A. Basic Biology, Physiology, and Nutrition

1. Mosquitoes. At Gainesville, Fla., studies were continued on the biology of Anopheles quadrimaculatus by artificially augmenting an isolated breeding area. It was shown that (1) The density of the population was increased in the study area when a constant breeding area was supplied to egg-laying females and when a blood source was provided, (2) Wild females preferred natural ponds as an egg-laying site but used artificial ponds when natural areas were not present, and (3) Anopheles quadrimaculatus adults preferred resting boxes which were painted black and placed on a horizontal plane.

New rearing diets and rearing techniques were evaluated for Anopheles quadrimaculatus. Rye grass infusion or extract accelerated larval development. There was a positive correlation between the number of larvae per rearing pan and larval mortality. Protozoans were an important source of food for the larvae. A rearing method incorporating all of these factors increased survival and size of the insects and decreased the time required for development and their tolerance to insecticides. Similar results were obtained with four other species of mosquitoes, Aedes aegypti, A. taeniorhynchus, Culex quinquefasciatus, and Aedes triseriatus.

A new method of separating pupae of Anopheles quadrimaculatus from larvae has been adopted. When mixtures of both are placed in ice-water, larvae sink and pupae float allowing rapid separation through the use of a funnel. Time required to separate the stages in colony production was reduced 86%.

At Corvallis, Oreg., studies were continued on the biology of the mosquito, Aedes increpitus in the Willamette Valley. During the winter months of the last three years, larvae of this species have been collected in numerous habitats of the flood plain of the Willamette River. Following an unusually protracted period of subfreezing temperatures during which a low of 8°F was registered and near-record floods occurred during which all low lying areas in the Willamette Valley were inundated for several days, larvae could be readily collected. The strain of increpitus in the Willamette Valley apparently has become well adapted physiologically to the rigors of the area over a long period of time. In other studies, a strain of Culex pipiens quinquefasciatus, which is orange in color as 4th-instar larvae and newly formed pupae, has been isolated. The strain has bred true for three generations and appears to be genetically recessive.

At Lake Charles, La., basic studies on the biology of floodwater mosquitoes was conducted. Studies have shown the comparative longevity, blood-feeding, and oviposition patterns of different species of flood-water Aedes species. Aedes taeniorhynchus, A. sollicitans, and A. infirmatus are more important as pest mosquitoes than other species that occur in the area. Studies on the amount of blood ingested by 12 pest mosquito species occurring in Southwestern Louisiana indicated that females of all species ingested sufficient blood at one feeding to at least double their body weight. Psorophora cyanescens (Coquillett), Aedes atlanticustormentor, and Anopheles quadrimaculatus Say, more than tripled their body weight with blood from one feeding.

Research has been conducted under two contracts at the University of Southwestern Louisiana and McNeese State College. Light trap collections have shown the production, relative abundance and dispersal of pest mosquitoes in the Gulf Coast area of Louisiana. Data has been maintained on rainfall and temperature in relation to mosquito production and some information has been obtained on the influence of rains versus tidal action in mosquito production. An impoundment is being developed to study the effects of impounding and water management procedures on mosquito production in the area.

2. House flies. At Gainesville, Fla., research was continued on basic biology of the house fly. Evidence was obtained that an olfactory attractant, or pheromone, specific for the males of Musca domestica L. is not produced only by females. The attractant was found on contaminated holding cylinders and on dead and non-virgin females. In addition, live males were also somewhat attractive. The degree of attraction was of a low order, resembling other reported sex pheromones of the house fly. This attractant was soluble in methane and slightly soluble in benzene. Data also showed that the time of day at which pupal eclosion occurs is influenced by photoperiod, but photoperiod may not be the only controlling factor. Response to insecticides was also shown to be regulated by photoperiod.

In mating experiments, female house flies mated more readily with males from their own strain than with those of other strains. When normal females from the laboratory or Grand Turk (wild) strains were confined with normal males from one strain and chemosterilized males from the other, they mated more readily with males of their own strain, whether sterilized or not. Chemosterilized males competed more successfully than normal males of the same strain.

At Corvallis, Oreg., research was conducted on the genetics and physiology of house flies and data developed in these studies were used to elucidate mechanisms of insect resistance to insecticides.

Several mutants of the house fly were isolated from normal and gamma-irradiated strains reared in the laboratory. Several of the mutant strains have been defined genetically and are being maintained. Most mutants



involved wing form, wing positioning, or pattern of wing venation. Three established mutant strains, classic wing, stubby wing, and dot vein have proved useful in the genetic analysis of insecticide resistance. For example two DDT resistant strains of house flies were found to possess a fifth chromosomal dominant which confers moderate resistance to DDT, but does not confer resistance to o-chloro DDT. In addition, one of the strains possessed a second chromosomal recessive which conferred moderate resistance to DDT and high tolerance to o-chloro DDT. The presence of both factors in a strain confers virtual immunity to DDT. Substrains were isolated, each possessing only one major factor for resistance and the nature of the two independent factors for resistance confirmed through appropriate crosses and bioassays. Resistance associated with the fifth chromosomal dominant is that for DDT dehydrochlorinase. The mechanism of resistance associated with the second chromosomal recessive is unknown, but apparently does not involve dehydrochlorination.

Physiological studies of mechanisms of resistance to organophosphorus insecticides in house flies showed that blocking of Ali-E with a selective inhibitor increased the accumulation of paraoxon and also the toxicity of parathion and paraoxon in both susceptible and parathion-resistant strains. These results indicated that Ali-E is an important detoxifying enzyme. The role of Ali-E in organophosphate poisoning appeared to be related more to detoxication of paraoxon than of parathion in both susceptible and parathion-resistant house flies.

A resistance factor (esterase(s)) for parathion in house flies was shown to be transmitted in a dominant manner and was at least 1000 times less sensitive to inhibition by paraoxon than a corresponding esterase present in a susceptible marker strain.

In other studies house flies kept in complete darkness from the time of emergence from pupae to separation of the sexes as adults mated successfully. Sex ratios were normal among the offspring.

In Oregon extensive studies were continued on the biology of the little house fly (Fannia canicularis). Investigations showed that unmated Fannia would deposit a few normal-appearing eggs, but none hatched. Most of the eggs were laid after the flies were three to four weeks old, while oviposition in the mated flies began in eight or nine days.

A standard CSMA medium used to rear the little house fly was treated with a  $P_{32}$ -labeled  $H_3PO_4$  solution, the fly larvae being transferred to the treated medium when they reached the third larval stage. Larvae, pupae, puparia, and adults were well labeled when exposed in media containing 1  $\mu$ c/larva, but not at 0.1  $\mu$ c/larva. Much higher levels of radioisotope labelling were obtained at 5 and 10  $\mu$ c/larva, but the 10  $\mu$ c/larva medium caused undue mortality.

3. Lice and Mites. In Oregon, studies showed that colonies of the northern fowl mites on white leghorn chickens declined during early summer each year, suggesting that the mites aestivate during the warmer months. Studies to confirm this failed as mite populations also declined on chickens held at 50° to 66°F. with 10 hours of illumination.

Studies were initiated in Oregon to determine the susceptibility of Japanese quail to poultry ectoparasites. This quail is now being used in research at numerous government, industrial, and academic laboratories as an experimental animal. Quail were exposed to heavy numbers of the northern fowl mite for four weeks, but infestations failed to develop. Similar results were obtained with chicken body lice (Menacanthus stramineum) over the 4-week period.

## B. Insecticidal and Sanitation Control

1. Mosquitoes. At Gainesville, Fla., the developmental program on insecticidal compounds for control of mosquitoes was continued. A large number of new candidate materials were tested in the laboratory for their potential as larvicides and adulticides; many of which proved promising for further development.

Tests were conducted to evaluate fogs of naled, fenthion, Bayer 39007, and malathion against caged salt-marsh mosquito adults. Bayer 39007 was the most effective in these tests followed by fenthion, naled, and malathion. A field test in which different formulations of malathion were applied by airplane at a ratio of 0.05 lb/acre showed a reduction of 81% in population levels with the fog oil formulation, 76% with fuel-oil formulation, and 44% with water emulsions. Airplane spray tests with four organophosphorus insecticides on adult salt-marsh mosquitoes indicated all were highly effective at low dosage rates. Comparison of the effectiveness of aerial sprays of malathion applied as a thermal fog and as a fuel oil spray showed the latter to be more effective.

Tests were conducted in the rice-growing area near Stuttgart, Ark., to evaluate the residual effectiveness of some new insecticides against natural infestations of Anopheles quadrimaculatus. The insecticides were applied to the walls and ceilings of farm buildings at 200 mg/ft<sup>2</sup> as wettable powders, or emulsions or both. Pre- and post-treatment counts were made of the mosquitoes resting in the treated buildings as well as in six untreated buildings which were utilized as checks. An emulsion of Hercules 9485 was highly effective, causing 99.7 to 100% reductions for at least seven weeks. As this compound was not available at the beginning of the series, it was applied from two to three weeks after the other treatments. A wettable powder formulation of Shell SD-8530 caused reductions of 98 to 100% for 9 to 10 weeks. A malathion wettable powder used as a standard caused 100% reductions for four weeks and 96 to 100% reductions throughout the 9-week test period. Hercules 9326 emulsion caused 100% reduction of the mosquito infestations for at least 9 to 10 weeks in two buildings but



in a storage shed produced only 91% to 96% control in the sixth and eighth weeks. Wettable powders of CELA S-1942 and CELA S-2225 were slightly less effective, with control falling below 70% in some buildings by the sixth week. Shell SD-8211 was highly effective in two buildings but not in a third.

Tests were also conducted to evaluate the residual effectiveness of treated cheesecloth when applied to the walls and ceiling of buildings in the same area. The cheesecloth, which was purchased in rolls 3 feet wide, was first flameproofed and then impregnated with Bayer 39007. Buildings in which a complete coverage of treated cheesecloth had been used showed 100% reduction of the mosquito populations for the full 10-week duration of the test. All buildings treated by means of a strip of cloth around the edge of the ceiling and in the corners showed 100% reduction for five weeks, and 82 to 99% control for the next five weeks. Buildings treated by means of cloth around the edge of the ceiling only, or in the corners only, showed 98 to 100% reduction of mosquitoes for five weeks, and 84 to 100% control for the next five weeks.

At Corvallis, Oreg., tests were continued on the development of more effective insecticides for mosquito control. In field tests against snow-water Aedes mosquito larvae, excellent results were obtained with lindane, BHC, and fenthion at 0.05-0.1 lbs/acre. Abate and Dursban were generally less effective. Against mosquito breeding in log ponds, granular formulations of fenthion and abate gave excellent control. Both were also effective when applied with a pump oil can. In cooperative tests in California low volume airplane sprays of malathion and fenthion showed considerable promise as mosquito larvicides.

Infusions and hot water extracts made from several tree species were tested for toxicity against Culex tarsalis larvae. Toxic elements were found in Western red cedar, ponderosa pine, and to a less extent in lodgepole pine and redwood. Similar hot water extracts made from Douglas fir, Sitka spruce, Western hemlock, big leaf maple, red alder, and white fir were nontoxic to larvae. Studies are in progress to characterize the toxic principles through fractionation of extracts.

None of seven analogs of DDT showed promise against resistant Culex tarsalis larvae.

At Corvallis, Oreg., experiments with  $C^{14}$ -TDE indicated that both susceptible and DDT-resistant Culex tarsalis larvae detoxified TDE by dehydrochlorinative and oxidative routes. The results suggested that resistance to DDT and related compounds in tarsalis involves a mechanism other than dehydrochlorination.

Studies were continued in the search for compounds that would act as synergists to overcome insecticide resistance in mosquitoes. Of a number

of phosphorus esters, butyl-containing esters were most effective although other types showed activity.

2. House fly. At Gainesville, Fla., research was conducted on the development of safer, more effective insecticides. Materials were evaluated in the laboratory as contact sprays and residual toxicants as a basis for selecting promising insecticides for field evaluations. Evaluations as residual toxicants included different formulations of the materials. Twenty-one promising compounds were tested as house fly larvicides in manure under caged poultry. Four compounds were highly effective as larvicides.

Residual tests were conducted with emulsions of malathion, diazinon, ronnel, dimethoate, naled, fenthion, and Bayer 41831, and with wettable powders of malathion and Mobil MC-A-600 against house flies in barns. All were applied at 100 mg/ft.<sup>2</sup> Control was considered satisfactory as long as the reduction produced by the chemical was 75% or above. Dimethoate residues gave satisfactory control on most occasions for 14 days, after which they were ineffective. Mobil MC-A-600 wettable powder gave satisfactory control for 14 days in one test, but failed as early as the first day in a replication of the test. Other compounds gave satisfactory control for shorter periods.

At Corvallis, Oreg., research was continued to find compounds effective in synergizing organophosphorus insecticides and resistant strains of house flies. A number of different types of phosphorus esters were effective when combined with either malathion or parathion in overcoming resistance in house flies to these two compounds. Materials synergizing malathion against resistant insects differed considerably from those known to potentiate the toxicity of malathion to mice or to cause ataxia in poultry.

In Texas, dichlorvos impregnated resin strips were tested in poultry houses to determine whether control of the house fly could be obtained in this manner. Each of two 10 x 10 ft. poultry houses contained 16 white leghorn layers. Both had windows on two sides to provide cross-ventilation. The windows were kept closed in one of the poultry houses; in the other, both windows were open the first two weeks, then one was closed for two weeks, and finally both windows were closed for the final six weeks of the test. Poultry manure had been allowed to accumulate for two weeks prior to the tests. In the poultry house with both windows closed, two resin strips, containing 20% of dichlorvos were placed, one at each end of the layer unit, about four inches below the end. In the poultry house with windows opened for a part of the test, two similar strips were placed about 12 inches above each end of the layer unit. In the closed poultry house, 100% elimination of house flies was obtained in about 10 days; control of the flies remained at the 100% level for the next eight weeks. In the other poultry house there was no measurable control of flies. In a different test, 2 pounds of 4.4% Zytron granules per 100 ft<sup>2</sup> of manure under caged layer failed to reduce house flies more than the first two or three days after treatment. Larvae were present in the manure at the time of test, and the manure had been allowed to accumulate for two weeks.



Wind tunnel tests in Oregon showed adult little house flies (Fannia canicularis) to be susceptible to malathion, parathion, and two commercial candidate insecticides (Mobil MC A-600 and Shell SD-8436), but not to carbamate or DDT at the concentrations tested (up to 1.0% and 3.0%, respectively). Parathion and Shell SD-8436 were most effective, killing half of the flies at a concentration of 0.1%. Topical applications of heptachlor, malathion, and ronnel indicated no increase in resistance to these compounds with either of two Oregon laboratory strains.

3. Lice and Mites. In Oregon, further tests with the Hansen strain of the northern fowl mite indicated that Shell SD-8436 and Hercules 5727 were extremely toxic to the mite. The LD-50 for the former was 0.0005% and for the latter, 0.00025%; the LD-50 for malathion is about 0.0072%.

Field tests were continued in Texas to improve methods of controlling poultry lice. Litter treatments with 5% ronnel granules at 0.5 lb/100 ft<sup>2</sup> of litter reduced louse populations from 62 to 83% over a 37-day period after the first day when very little reduction was evident. At 1 pound of the granules per 100 ft<sup>2</sup>, louse reduction ranged from 91 to 98%. Two applications of the 0.5 pound dosage, applied 12 days apart, were just as effective, reducing lice over a range of 93 to 98% for the 37-day period of the test. Applied at 1 lb/100 ft<sup>2</sup>, 2% CELA S-1942 was less effective though the control increased from 55% the third day after treatment to a maximum of 90% on the 28th, 34th, and 37th day. The most effective material, 4.4% Zytron granules, gave 100% reduction of the lice within a week. A dust containing 8% of Imidan also gave 100% reduction of the lice within a week. When the Zytron granules were used to treat under cages of caged layers at 2 lb/100 ft<sup>2</sup>, no louse control was obtained, even when the initial dosage was repeated five weeks later. Thus, there is no fumigant effect from this insecticide. In further litter treatment tests, dimetilan, Imidan, and carbophenothion, were applied at 1 pound of 2-percent dust/100 ft<sup>2</sup>. Over the 28-day posttreatment period, reduction with dimetilan fluctuated, but never exceeded 87%; Imidan and carbophenothion gave 100% reduction of the lice. Resin pellets containing 5% of dichlorvos, applied at 0.25 and 0.5 lb/100 ft<sup>2</sup> of litter was relatively ineffective in controlling lice. At 0.25 lb/100 ft<sup>2</sup>, louse control ranged from 30 to 84% over the 28-day test period; at 1/2 pound, control ranged from 58 to 88% over the pretreatment counts. However, resin strips containing 20% of dichlorvos completely eliminated lice in a closed 10x10 ft poultry house when one strip was hung at each end of a caged layer unit. When each of the two windows (cross-ventilation) in a second caged layer house were left open for two weeks, with one window then closed for two weeks, and finally both windows closed for six weeks, no measurable louse control was obtained with these strips. The recommended malathion dip for lice contains 0.5% of malathion; two dips in 0.1% malathion, 17 days apart, completely eliminated a light infestation of lice in a small flock of white leghorn hens.

4. Fleas and ticks. Two dips, 17 days apart, in 0.1% malathion emulsion failed to control sticktight fleas in a small flock of white leghorn hens

in Texas. The house and yard were not treated and the fleas averaged over 150 per bird, before and after treatment. This is, of course, a very low concentration of malathion; the recommended concentration for dipping for control of lice and mites is 0.5% malathion. Two weeks later, the same flock was dipped in 0.5% carbaryl suspension and the poultry house floor treated with 5% carbaryl dust at 1 lb/40 ft<sup>2</sup>. This is the dust dosage recommended for control of lice and mites, but there is no recommended carbaryl dip for poultry. In spite of this treatment, the number of fleas per bird was only reduced from 160 to 50, and the count remained 50 per bird for the next 30 days. Single treatments of 5% dichlorvos resin pellets at 0.25 and 0.5 lb/100 ft<sup>2</sup> of litter (sawdust) gave excellent control of sticktight fleas and fowl ticks. Before treatment, populations of sticktight fleas varied from 80 to 100 per bird; fowl ticks varied from 8 to 10 per bird. On the 28th day after treatment, numbers of fleas and ticks (larval) were less than one per bird.

### C. Insecticide Residue Determinations

1. Residue studies. In Texas, studies were conducted to determine whether Zytron granules applied under the cages of chickens for fly control would cause residues to be stored in the body tissues of the chickens or in the eggs. The insecticide was applied at the rate of 2 pounds of 4.4% Zytron granules per 100 square feet. Chickens were sacrificed 1, 2, 3, and 4 weeks after treatment, then the premises were retreated and chickens sacrificed one week after the retreatment. Samples of eggs also were taken at each sampling date. No detectable residues were found in the eggs or in samples of fat, breast meat, thigh meat, skin, or liver on any of the sampling dates. (Limit of sensitivity of analysis 0.005 ppm). In a second test Zytron granules were added at the rate of 1 lb/100 ft<sup>2</sup> to the litter in houses where chickens were kept. Samples taken two and four weeks after this treatment showed detectable residues of Zytron ranging from 0.18 to 0.199 ppm in the fat, 0.007 to 0.133 ppm in the skin, and 0.020 to 0.025 ppm in the eggs. No residues were detected in the breast meat, thigh meat or liver.

2. Toxicity Studies. Research was conducted in Texas in cooperation with the Animal Disease and Parasite Research Division on the acute and chronic toxicity of insecticides and other chemicals.

Studies showed that the polyfunctional alkylating agents apholate, tepa, and metepa, which are insect chemosterilants, injected into the yolk sacs of developing embryos after varying periods of incubation, induced congenital abnormalities in embryos that survived to the 18th day of incubation.

Doses of each compound at 250 µg and higher per egg were lethal to 4-day old embryos. Death usually occurred within 72 hours. Each compound at 125 µg. per egg was lethal to 1- and 2-day old embryos in 72 to 96 hours but allowed 4-day old embryos to continue to develop for as long as 11



days before they died. Doses of each compound at 5.4 to 25 µg. per egg usually permitted embryos to develop. Each compound induced similar congenital abnormalities such as defects of the beak, eyes, digits, and legs; cerebral and visceral hernia; edema; growth retardation and reduced weight.

Seven insecticides were studied in 280 chickens 8 to 9 weeks of age to determine the oral toxicity. The maximum nontoxic dosages found were: coumaphos, 2.5 mg/kg, diazinon and dichlorvos, 5 mg/kg, Ciodrin and Ruelene, 100 mg/kg, and dioxathion, 250 mg/kg.

#### D. Biological Control

1. Mosquitoes. At Lake Charles, La., research on pathogens of mosquitoes has been conducted. Field collections throughout the area have shown infections of microsporidia in thirteen species including the genera, Culex, Aedes, Culiseta, Anopheles, and Orthopodomyia. Aedes grossbecki and Orthopodomyia signifera were new host records for a microsporidian. Spore sizes were determined and classification of the microsporidia studied. Transovarial transmission of microsporidian infections was studied in 12 species of mosquitoes and demonstrated in seven of the species.

The fungus Coelomomyces was found in field collections infecting larvae of Culex restuans, C. salinarius, Aedes vexans, A. sollicitans, and Culiseta inornata. Culex salinarius, C. restuans and Aedes sollicitans represent new host records for Coelomomyces. Field infection levels varied from very low to over 50 percent.

A polyhedral virus was reported from larvae of Aedes vexans and Psorophora ferox. Both species were previously unreported as hosts of viruses. Infection levels in the field were very low. A very lethal bacteria was collected from larvae of six mosquito species.

At Gainesville, Fla., two species of Thelohania were found in A. quadrimaculatus, one infecting the adipose tissue and the other the oenocytes. One species was found infecting the oenocytes of A. crucians larvae.

#### E. Insect Sterility, Attractants, and Other New Approaches to Control

1. Mosquitoes. At Gainesville, Fla., studies were continued on factors affecting the attraction of mosquitoes to their hosts and factors affecting the protection time from mosquito bites afforded by repellents. A large olfactometer was developed to study these factors as well as evaluate the efficacy of various attractant materials or factors.

The effort to develop effective space and systemic repellents was continued and slightly expanded. To date several materials show some space repellency to mosquitoes in that they prevent mosquitoes from penetrating 4-mesh

screening. Materials exhibiting some systemic repellency were found and further tests will be made on these materials.

Studies were continued at Gainesville, Fla., to evaluate materials as chemosterilants for mosquitoes and to evaluate the sterility principle of mosquito control. Tests with hempa as a chemosterilant indicated it would have little value as a residual sterilant for mosquitoes. Further selection and studies were conducted with the apholate-resistant colony of Aedes aegypti to clearly define the degree of resistance. This colony is at least 10 times as resistant to the sterilizing effects of apholate as the unselected, parent colony. Selections to increase resistance will be continued.

A sterile male release study was made in a semi-isolated area with Anopheles quadrimaculatus. This area was made more favorable by increasing the number of breeding sites and introducing additional wild stock of this mosquito to populate the area. When sterile males of wild stock were released in this area, sterility of the natural population increased from a very low degree up to 42%. When the releases were changed to sterile colony males the sterility in the natural population decreased confirming earlier work showing behavior differences between colony and wild strains in seeking out wild females. Apparently a sufficient number of males was not released to reduce the population levels of A. quadrimaculatus.

Studies were continued on the evaluation of chemosterilants for mosquitoes at Corvallis, Oreg. The chemosterilant, hempa, was not highly effective in sterilizing Culex tarsalis larvae in that rates as high as 200 ppm were required. As a residual treatment in glass jars, 10 mg of hempa per square foot sterilized adult males completely, but adult females only partially. In wind tunnel tests against adults, a concentration of 10% caused high sterility, whereas a 5% spray caused only partial sterility and none was caused at 1% or lower. Males were generally more susceptible than the females.

At Corvallis, Oreg., studies were continued on sex and ovipositional attractants for mosquitoes. Preliminary tests indicated the presence of sex attractant in Culex quinquefasciatus. Further tests did not confirm this, nor the presence of a sex attractant in Culex tarsalis. Many mosquitoes are known to choose specific types of water for oviposition. Studies have shown that odors from grass infusions and log pond waters collected in distilled water were attractive to gravid females of Culex quinquefasciatus. These odors were not attractive to females of C. tarsalis. However, log pond water itself was more attractive to this species than either distilled water or distilled water plus log pond odors. Distilled water saturated with methane was also attractive to gravid females of C. quinquefasciatus but not to those of C. tarsalis. Gravid females of both species were more attracted to distilled water treated with 25 ppm of furfural than to water treated with 5 or 50 ppm.



2. House Flies. At Gainesville, Fla., research was continued on the development of chemosterilants and the sterility principle of control for house flies. Several hundred new candidate compounds were evaluated in primary screening and secondary development tests and many were found to exhibit sterilizing efficacy against both males and females. Particular attention was paid to evaluating two compounds--hempa and hemel--as sterilants by several routes of administration. These two materials will sterilize both sexes of the house fly.

Two series of field tests were conducted at farms in Florida to evaluate the effectiveness of two chemosterilants, hempa and apholate, for the control of house flies. At the farm treated with hempa, house flies were reduced in abundance from 71 per grid to 0 within eight weeks. Grid counts remained at zero for the remainder of the test period. At the farm treated with apholate, population levels decreased from 200 per grid to less than 10 within six weeks and remained constant at a low level throughout the remainder of the test.

Ninety chemicals were screened as chemosterilants against adult house flies. Six compounds were toxic and 10 reduced the fertility to some extent in fly food or sugar. Thirty-two compounds, previously shown to sterilize house flies, were also tested again at higher or lower concentrations.

Tests were conducted with 21 compounds to determine their effectiveness as male house fly sterilants. Of the 14 chemicals Olin 53330 (ENT-50838), Squibb Olin 53361 (ENT-50839), Squibb Olin 53356 (ENT-50840), and Squibb Olin 53263 (ENT-50842) which sterilized at a concentration of 0.05% in the sugar diet. Sankyo Co. RES-101 (ENT-50845) induced sterility at this dosage in sugar in the first two eggings.

Basic studies were continued on the cytological effects of chemosterilants on house fly reproductive systems and previous sectioning, fixing, and staining techniques have been used to study several new chemosterilants.

Olfactometers designed by Gouck and Schreck were used to initiate a search for more effective house fly attractants. Nineteen compounds were tested, using Edamin as a standard. Beef protein concentrate was attractive to females but not to males. The other materials were not as effective as Edamin.

At Corvallis, Oreg., research was conducted on chemosterilants and attractants for the house fly and the little house fly. With the little house fly, hempa caused sterility as a residual deposit on glass at 50 mg/ft<sup>2</sup>. However, the sterilizing dose caused some fly mortality. Higher doses were highly toxic to the adult flies; lower doses did not sterilize. When fed orally in the adult food to the little house fly, hempa was toxic at 0.25% and lethal at 1.0%. High, but incomplete sterility was caused at concentrations as low as 0.01%. Topical treatments of hempa and hemel sterilized males of the little house fly without causing mortality, but

not the females. In general treatments causing a high degree of sterility did not affect the mating competitiveness of females. Four known anti-oxidants exhibited little effect on egg production or egg hatch with the little house fly. Dosages of gamma radiation greater than 1000 r given to pupae of the little house fly prevented oviposition by emerging adults.

At Corvallis research was continued on the sex pheromone in house flies. The presence of a low titre of pheromone in extracts of pupae and young females was shown and confirmed. Higher activity of the pheromone was demonstrated in 3-day-old flies. The presence of the pheromone was shown in female flies from strains of different origin. Males of different strains reacted to extracts from females of different strains, though differences in behavior were apparent.

At Corvallis, Oreg., further studies showed that the sex pheromone in female house flies increased with the age of the flies, with the greatest increase occurring on about the third day after emergence. Comparative tests with benzene extracts of female house flies showed that high concentrations applied to pseudo flies inhibited male response. Similar inhibition occurred when normal extracts were applied to large surfaces within test chambers. Comparative tests with extracts of females with different solvents showed a much greater amount of the pheromone in hexane than in other solvent extracts. Efforts are being made to determine the chemical nature of the pheromone.

In Oregon studies were continued with the little house fly, Fannia canicularis. In mating experiments with radioactive flies (reared in a medium treated with  $P_{32}$ -labeled  $H_3PO_4$  solution) crossed with normal flies, eggs from pairings with flies exposed to 5  $\mu$ c/larva were only 18.3% fertile; those exposed to 10  $\mu$ c/larva did not oviposit, and untreated flies showed 88% fertility in eggs deposited. In other tests, late stage pupae of the little house fly were irradiated in a Cobalt 60 source at levels from 1,000 r to 10,000 r. Radiation dosages of 2,500 r and above totally arrested oviposition. At 1,000 r oviposition was reduced and egg hatch was only 20% of normal.

In studies with chemosterilants in Oregon, residues of 20 mg of the new chemosterilant, hempa, per square foot were only partially effective in sterilizing the little house fly. Residues of 50 mg/ft<sup>2</sup> gave essentially complete sterility of adult flies exposed, but mortality of the flies was high. Male flies fed 0.1% hempa in honey-water for 4 days were fully competitive with normal males, reducing the fertility of eggs laid by females by the expected amounts in a single, preliminary test. To determine the amount of hempa and the related chemosterilant, hemel, required to sterilize the little house fly, topical treatments were made. With either compound, about 24 micrograms per fly sufficed to sterilize the males. About 96 micrograms of hempa were required to sterilize the females, a dosage that caused high mortality. Hemel could not be tried above 40 micrograms per fly, due to solubility problems; this dosage failed to



sterilize the females. Both materials sterilized the males at a dose that caused little or no mortality. The flies used weighed an average of 8 milligrams apiece. Studies are being initiated to determine whether the little house fly can develop resistance to chemosterilants. In preliminary tests, a reduced hatch resulted in flies fed 0.001% hempa (78 to 62.5% hatch). Chemosterilants break down quickly on exposure. Four antioxidant compounds, under consideration as a means of reducing this breakdown, were screened to be certain they had no sterilizing action of their own. When fed at 0.1% in honey and water, none affected egg production or egg hatch.

Tests to discover attractants for the little house fly continued in Oregon. To provide a more accurate record of the duration and frequency of visits by flies on colored strings, lengths of white twine were treated with aqueous radiophosphorus solution. A consistent level of radioactivity was not induced in flies until small amounts of sugar or honey were added to the radiophosphorus solution. When this was done and the solution standardized at 50 µc/ml, effective labeling of the flies occurred in 24 hours.

In Maryland, the effectiveness of electrocutor-grid screens placed in windows plus an indoor electrocutor trap with black light lamps for controlling fly populations was evaluated in two calf barns where large numbers of house flies and stable flies were present. Although many flies were killed by the grids, no substantial reduction of the fly population within the grid-screened barn could be measured unless all major openings were closed or screened. When the animals were allowed access to exercise lots through open doors, the fly populations in the test barn and the "check" rapidly equalized.

#### PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

##### Basic Biology, Physiology, and Nutrition

- Dame, David A., and Richard L. Fye. 1964. Studies on the feeding behavior of the house fly, Musca domestica L. J. Econ. Entomol. 57: 776.
- Gahan, James B., and Carroll N. Smith. 1964. Problems connected with raising mosquitoes in the laboratory. Bull. World Health Organ. 31: 445-448.
- Gjullin, C. M., Thomas D. Mulhern, and Richard C. Husbands. 1963. The daily resting cycles of several species of mosquitoes. Mosq. News 23: 203-210.
- Hoyer, Richard F., Frederick W. Plapp, Jr., and Richard D. Orchard. 1965. Linkage relationships of several insecticide resistance factors in the house fly (Musca domestica L.). Entomol. Exptl. Appl. 8: 65-73.
- Morgan, Philip B., and G. C. LaBrecque. 1964. Effect of tepa and metepa on the ovarian development of house flies. J. Econ. Entomol. 57: 896-899.
- Morgan, Philip B., and G. C. LaBrecque. 1964. Preparation of house fly chromosomes. Ann. Entomol. Soc. Amer. 57: 794-795.
- Murvosh, Chad M., R. L. Fye, and G. C. LaBrecque. 1964. Studies on the mating behavior of the house fly, Musca domestica L. Ohio J. of Sci.: 264-271.

- Murvosh, Chad M., G. C. LaBrecque, and Carroll N. Smith. 1965. Sex attraction in the house fly, Musca domestica L. Ohio J. of Sci. 65: 68-71.
- Plapp, Frederick W., Jr., Walter S. Bigley, Darrell I. Darrow, and Richard F. Hoyer. 1965. Esterase activity in mosquitoes and its possible relationship to organophosphate and carbamate resistance. Mosq. News 25: 30-35.
- Rogoff, W. M. 1965. Mating of the house fly, Musca domestica L., in monitored darkness. J. Med. Entomol. 2: 54-56.
- Weidhaas, D. E., D. B. Woodard, C. H. Schmidt, and H. R. Ford. 1964. Biology of Anopheles quadrimaculatus under field conditions in Central Florida. Ann. Entomol. Soc. Amer. 58: 145-150.

### Insecticidal and Sanitation Control

- Darrow, Darrell I., and Gaines W. Eddy. 1964. Laboratory evaluation of sprays of selected insecticides against the mosquito Culex tarsalis Coq. Mosq. News. 24: 218-220.
- Gahan, James B., and A. Nelson Davis. 1965. Toxicity of a group of new insecticides to Aedes taeniorhynchus. Proc. of 51st Ann. Mtg. of New Jersey Mosq. Ext. Assoc.: 162-165.
- LaBrecque, G. C. 1964. House flies and their control. Proc. 17th Ann. Alabama Pest Cont. Conf.: 19-24.
- LaBrecque, G. C., H. G. Wilson, and James B. Gahan. 1965. Residual effectiveness of some insecticides against adult house flies. USDA, ARS 33-103.
- Plapp, F. W., Jr., G. A. Chapman, and G. W. Eddy. 1963. Synergism of malathion against resistant house flies and mosquitoes. J. Econ. Entomol. 56: 643-649.
- Plapp, F. W., Jr., G. A. Chapman, and W. S. Bigley. 1964. A mechanism of resistance to Isolan in the house fly. J. Econ. Entomol. 57: 692-695.

### Biological Control

- Kellen, William R., Harold C. Chapman, Truman B. Clark, and James E. Lindegren. 1965. Host-parasite relationships of some Thelohania from mosquitoes (Noxematildea: Microsporidia). J. Invert. Pathol. 7: 161-66.

### Insect Sterility, Attractants, and Other New Approaches to Control

- Fye, R. L., H. K. Gouck, and G. C. LaBrecque. 1965. Compounds causing sterility in adult house flies. J. Econ. Entomol. 58: 446-448.
- Gjullin, C. M., and Jessup O. Johnsen. 1965. The oviposition responses of two species of Culex to waters treated with various chemicals. Mosq. News 25: 14-16.
- Glancey, B. Michael. 1964. A review of the current status of autocidal methods for mosquito control. Proc. 35th Ann. Mtg. Florida Anti Mosq. Assoc.: 22-25.
- Gouck, H. K., and G. C. LaBrecque. 1963. Compounds affecting the metabolism of house fly larvae. USDA, ARS 33-87.
- Gouck, H. K., and G. C. LaBrecque. 1964. Chemicals affecting fertility in adult house flies. J. Econ. Entomol. 57: 663-664.



- Gouck, H. K., and C. E. Schreck. 1965. An olfactometer for use in the study of mosquito attractants. J. Econ. Entomol. 58: 589-590.
- LaBrecque, G. C. 1963. Chemosterilants for the control of house flies. Advances in Chem. 41: 42-46.
- LaBrecque, G. C. 1964. Status of research with chemosterilants. Proc. Assoc. of Southern Feed, Fertilizer, and Pesticide Cont. Offici.: 69-71.
- Lewallen, Lawrence L., Harold C. Chapman, and William H. Wilder. 1965. Chemosterilant application to an isolated population of Culex tarsalis. Mosq. News. 25: 16-18.
- Schmidt, C. H., D. A. Dame, and D. E. Weidhaas. 1964. Radiosterilization vs. chemosterilization in house flies and mosquitoes. J. Econ. Entomol. 57: 753-756.

## II. UTILIZATION RESEARCH

### POULTRY - PROCESSING AND PRODUCTS

Western Utilization Research and Development Division, ARS

Problem. The \$1.6 billion poultry meat industry operates on very narrow profit margins. In order to keep abreast with developments in the modern food industry, poultry must be converted into a wide variety of products having high quality and improved convenience, at costs attractive to consumers and remunerative to the poultry grower. More information on the properties and processing of poultry is needed to enable us to better utilize poultry in a variety of forms attractive to consumers. Increased utilization of poultry would also serve toward eliminating our feed grain surplus, increasing returns to farmers and providing better products for American consumers.

Although poultry is an efficient converter of feed to meat, more grain is used by poultry per calorie of food produced than by any other commercial animal because a high percentage of the poultry diet is grain and because poultry meat contains exceedingly little fat. Furthermore, one-fourth of all grain fed to animals is used for poultry and egg production. Hence, increased consumption of poultry products would be an effective means of increasing markets for surplus grain. Also, the efficiency of feed utilization by poultry makes possible low prices within reach of more consumers. A still further benefit would arise from the increased use of poultry by improving the nutrition of consumers having diets now low in animal protein.

The consumption of poultry has steadily increased from a 1947-1949 average of 22 lbs. per capita to 39 lbs. for 1963. This important increase has involved price, quality of product, availability, and disposable income. Because of the current low profit margin it is impractical to increase consumption by lowering farm prices. Increased demand for and consumption of poultry will require higher quality and more convenient products and a greater variety to meet the desires of the modern consumer. However, in addition to greater returns from increased demand, a greater profit margin for the farmer can, of course, come from greater efficiencies in processing.

The trend toward convenience foods and further processing has primarily led to precooked poultry products which are generally less stable, more subject to warmed-over flavors, and more likely to provide texture problems than uncooked items. With the expansion of operation and the emphasis on continuous, more efficient processing, need has arisen for improved processing procedures for feather removal, chilling, tenderization, freezing, deboning, and commercial cooking. Lowering the cost and improving the quality of products that can be stored at ambient temperatures, such as canned, dried, cured, and irradiated products, offer potential for poultry utilization in domestic and export markets. As a foundation for applied studies, further knowledge is needed on the chemical nature of flavor and flavor changes in processing and storage, on tenderness development, and on proteins, lipids, and other components.



## USDA AND COOPERATIVE PROGRAM

Basic and applied research on poultry meat and poultry meat products are conducted at the Division headquarters at Albany, California and by contract in Madison, Wisconsin, and Berkeley, California. Fundamental studies on poultry flavor are concerned with the identification of flavor precursor constituents in poultry meat and in the isolation and identification of volatile flavor components developed during the cooking of poultry. The chemistry of muscle protein and post-mortem chemical changes are investigated relative to the tenderness and other quality characteristics of poultry. The basic physiological character of feather release mechanism in fowls is studied to provide a foundation for improved feather removal. Applied research is conducted on the stability of cold-tolerant organisms; special problems of flavor, texture and stability of precooked frozen foods; and processing factors that influence tenderness of poultry meat.

The Federal program of research in this area totals 12.9 professional man-years, including contract research equivalent to approximately 1.7 professional man-years per year. Of this number, 4.3 are assigned to chemical composition and physical properties; 8.6 to new and improved food products and processing technology.

## PROGRAM OF STATE EXPERIMENT STATIONS

State stations conduct both basic and applied researches on poultry and turkey meat and meat products. One phase of this work is directed to solution of problems associated with maintenance of the quality of fresh poultry. The effects of processing procedures such as feather removal, dressing, chilling and packing conditions are related to the bacteriological, organoleptic and physical properties of the finished product. Factors such as wholesomeness, microflora, condemnation losses and biochemical changes during processing must be evaluated.

There have been extensive new developments in work methods, equipment and facilities, and processing and packaging procedures. For example, continuous chillers have reduced the chilling time, increased uniformity and effectiveness of chilling and improved broiler quality. Current work involves observation of bacterial counts, water uptake, leaching of solids from carcasses and organoleptic quality as well as shelf-life of carcasses chilled by this technique.

Fundamental studies are concerned with the chemical and physical properties of poultry and turkey meat. Chemicals that affect the chemical bonding within and between protein molecules are used to study their effect upon the tenderness of the muscles after cooking. Results, to date, suggest that sulfur bonding as affected by chemical agents may be a factor in the ultimate tenderness of poultry meat. Studies which seek to determine the specific pathways and effects of post-mortem changes are continuing.



The influence of dietary fat upon the composition of the chicken carcass and the effects of increased polyunsaturated fatty acid content of the meat are being carefully evaluated. Special attention is being given the biological value of the protein and other constituents.

Continued research effort is directed to evolving an understanding of the precursors of chicken flavor and of the components which make up chicken flavor. Advanced techniques of flavor analysis are being used.

Basic microbiological studies continue to be directed to establishing the source of organisms, and the natural flora on poultry and poultry products; to the effects of organisms found in bruised or diseased tissue; to the build-up of organisms during the various stages of processing; and to means for extending shelf-life by retarding bacterial growth in poultry and poultry products. Another phase of the microbiological program deals with growth, survival and control of potentially pathogenic organisms.

New product research centers on accumulating fundamental information basic to the development of new or improved products. Significant results have been obtained in establishing the time-temperature relationships for processing turkey rolls. New approaches are sought to improve form, texture, flavor, juiciness and quality of products. Special attention is being given development of new poultry items from lower grades of poultry including ways to improve and utilize the carcasses of aged fowl.

Other investigations are concerned with insecticide residues in poultry and the effect of cooking and storage time and temperatures upon residue levels. In addition, market tests and economic feasibility studies seek to determine the market potential for new or improved poultry items.

A portion of the research on poultry products is conducted under the regional project NCM-40.

The research effort devoted to increased utilization of poultry and turkey products is approximately 19.2 professional man years.

## PROGRESS -- USDA AND COOPERATIVE PROGRAMS

### A. Chemical Composition and Physical Properties

1. Post-Mortem Biochemistry and Tenderness. Basic investigations on the muscle chemistry of poultry are principally concerned with characterizing post-mortem changes in metabolites of the glycolytic cycle in relation to tenderization. An extremely labile metabolite, creatine phosphate, was previously shown to have a concentration dependent upon the conditions that prevail during slaughter. Electrically stunned birds had only one-eighth as much creatine phosphate as did birds that were anesthetized with pentobarbital prior to slaughter. Studies were extended to include determination of adenosine triphosphate. This compound was significantly higher for birds



that had been electrically stunned. The time required for adenosine triphosphate to drop to 50% of its resting level was 7 hours for anesthetized birds and 3 hours for stunned birds. Since rigor mortis occurs when the muscle adenosine triphosphate level is reduced approximately 50%, these experiments show how anesthetization can significantly delay onset of rigor mortis.

Contract research at the American Foundation for Biological Research in Madison, Wisconsin was initiated on the histology of structural changes in poultry muscle. Electron microscopy of pre-rigor and post-rigor muscle revealed wide structural changes that can be induced by heating and by freezing. Rapid freezing and thawing cause some deformation of striations in pre-rigor muscle, but no substantial change has been observed in post-rigor muscle. Heating results in a great disturbance of the striations of pre-rigor muscle and disorganization of certain muscle structures, but very much less change in post-rigor muscle. To the extent possible, muscles were held in restraint during the treatments to prevent the drastic shortening that occurs upon thawing and to lesser degrees with other treatments. Myofilaments in muscles remained in their original longitudinal position if restrained during thawing, but they aggregated laterally to form dense, irregular bands. The combination of freeze-thaw-heat applied to pre-rigor muscle accentuated the large, irregular bands caused by thaw rigor. The aggregation of myofilaments quite possibly affects toughness of meat.

2. Chemistry of Poultry Flavor. We reported previously that hydrogen sulfide ( $H_2S$ ) originates from the amino acids cystine and cysteins during cooking of chicken. The quantity of  $H_2S$  in freshly boiled, fried, or roasted chicken meat is sufficient to create an equilibrium  $H_2S$  partial pressure in air above the meat that is at least 4 to 34 times greater than the  $H_2S$  partial pressure at which the odor of pure  $H_2S$  in air can be recognized. Therefore,  $H_2S$  evidently contributes to the aroma of cooked chicken meat. However, the odor of  $H_2S$  cannot be recognized as a separate, distinct entity in this total aroma. Furthermore, the odor of  $H_2S$  over chicken broth was not recognized by an odor panel until enough  $H_2S$  had been added to it to cause an  $H_2S$  partial pressure of at least 36 microns, which is at least four times the level that is readily detectable in the absence of other odors. Evidently the characteristic odor of  $H_2S$  becomes masked or altered to a marked degree as it blends with other volatile components of cooked chicken.

The non- $H_2S$ -sulfur content of chicken fat was increased by bubbling pure  $H_2S$  through 5% solutions of carbonyl compounds in chicken fat. These samples had potent new aromas. This indicates that cooked chicken aroma may also include aromas formed by reaction of  $H_2S$  with carbonyl compounds.

More than 200 components are evident in chicken meat volatiles chromatographed on a 300 ft. x 0.01 inch capillary column. By combining gas chromatography with mass spectrometry, 6 sulfur compounds, 5 aldehydes, 8 methyl ketones, 5 benzene derivatives, 3 furan derivatives, and heptane have been tentatively identified in this mixture. Obviously we confront a major task in determining



the identity, origin, and flavor significance of more than 200 volatile compounds. The intractable nature of this task indicates that we need to evaluate the significance of groups or fractions of the volatiles in an attempt to simplify the problem. Fractions that show no aroma contribution will not be chemically characterized in detail.

3. Physiology of Feather Release. Contract research on characterization of the physiological mechanisms that control feather tightening and release in poultry has been concluded at Michigan State University. The direct effect of the central nervous system on the force required to pull feathers was established. Changes in pressure measured within the empty feather follicles indicate that the constrictive force exerted on the feather shaft is by the follicle wall. Anesthetics, specific nerve-stimulating or nerve-blocking drugs, and mechanical or electrical stimulation produced parallel effects on intrafollicular pressure and feather-pulling force. Increase in intrafollicular pressure and feather-pulling force induced by injection of nerve-stimulating drugs into anesthetized birds was reduced or neutralized by the action of specific nerve-blocking drugs. Basic information thus obtained can serve as a foundation for development of methods for feather removal that are not dependent upon a completely empirical approach.

#### B. New and Improved Food Products and Processing Technology

1. Freeze-Drying. Product-in-tube and fin-tube rotating freeze dryers are being studied to establish operating conditions required for continuous freeze-drying of cooked chicken meat and other products. (In-put and outlet reservoirs allow for limited continuous drying without breaking the vacuum of the system.) Drying rates up to twice those of conventional tray and shelf freeze-drying were attained over a range of physical conditions in the new equipment. A short hexagonal tube dryer capable of handling small batches of product was built and is being used to determine the tumbling freeze-drying characteristics of several classes of products. General design features of a commercial-size unit are being developed. A method is also being developed for tumbling freeze-drying of liquid products. Liquids are formed into frozen pellets by letting drops fall into a chilled fluorocarbon refrigerant; the pellets then can be freeze-dried in a tumbling bed under vacuum.

A commercial evaluation was made by a cooperator showing a very clear advantage for our continuous unit over any freeze dryers ever made before. The specific advantages are rapid drying time, simple equipment, steady labor demand, and steady refrigeration load. To exploit the last advantage, we are examining several ideas for a continuous condenser.

Contract research at the University of California, Berkeley, was initiated to develop engineering information on rate-controlling factors in freeze-drying and methods for programming heat input and determining drying end-points. Equipment has been designed for the continuous weighing of pieces of poultry meat during freeze-drying and for determining surface areas and pore sizes in freeze-dried products. The contractor is also studying heat transfer to cold liquids that are in contact with water vapor which freezes into the



liquid. This will assist us in choosing among several of our ideas for continuous condensers.

Sensory evaluations of the texture and tenderness of frozen and freeze-dried poultry meats were compared with objective measurements of shear-resistance and water-holding capacity. Dark muscle rehydrated poorly and was tougher and drier than light muscle, yet dark muscle had a greater water-binding capacity than did light muscle after dehydration. Studies of protein extractability of dark and light meats are providing basic information that may help explain qualitative differences.

2. Tenderness of Poultry Meat. A small trained taste panel and a large untrained panel were used to compare subjective evaluations of toughness of turkey meat with objective shear-force measurements. The trained panel could distinguish differences of 4 pounds shear force. They commented that, in general, samples above 15 pounds shear force were tough. The larger untrained panel indicated toughness at a shear force above 20 pounds. Eight hours aging time was adequate to achieve tenderness in turkeys if the turkeys had been thawed before roasting. These studies remove any doubt that may exist concerning the necessity for aging turkeys prior to freezing; this costly procedure must continue until we can provide new information upon which to base improved processing methods. An untrained panel of about 100 people evaluated toughness differences in 9-week-old chickens. At least 4 hours aging was found to be desirable, even for birds that are thawed before cooking. Two types of shear measurement were used, which correlated well with each other and with the evaluations of the untrained panel.

## PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Chemical Composition and Physical Properties

- deFremery, D., and Lineweaver, H. 1964. Early post-mortem chemical and tenderness changes in poultry. Proc. 1st Internatl. Cong. Food Sci. and Technol., London, Sept. 18-21.
- de Wijs, Hermine, and Jollés, Pierre. 1964. Cell walls of three strains of mycobacteria (mycobacterium phlei, mycobacterium fortuitum and mycobacterium kansasii). Preparation, analysis and digestion by lysozymes of different origins. Biochim. Biophys. Acta 83(3), pp. 326-332. 1/
- Jollés, P., Petit, J. F., Charlemaane, D., Salmon, S., and Jollés, J. 1964. Etude comparee de quelques lysozymes humains (comparative study of some human lysozymes). Symposium Internazionale sul Lisozima di Fleming, 3rd, Milano, pp. 1-5. 1/
- Mecchi, E. P., Pippen, E. L., and Lineweaver, Hans. 1964. Origin of hydrogen sulfide in heated chicken muscle. J. Food Sci. 29(4), pp. 393-399.
- Peterson, Ronald A., and Ringer, Robert K. 1964. Intrafollicular pressure in the feather follicle. Poultry Sci. 43(5), pp. 1210-1214. (Contract)
- Ryan, C. A. 1965. Chicken chymotrypsin and turkey trypsin. Part I. Purification. Arch. Biochem. Biophys. 110(1), pp. 169-174.
- Ryan, C. A., and Clary, John J. 1964. Some reactions of chicken chymotrypsin and turkey trypsin with substrates and naturally occurring protease inhibitors. Arch. Biochem., Biophys. 108(1), pp. 169-171.
- Ryan, C. A., Clary, John J., and Tomimatsu, Y. 1965. Chicken chymotrypsin and turkey trypsin. Part II: Physical and enzymic properties. Arch. Biochem. Biophys. 110(1), pp. 175-183.

New and Improved Food Products and Processing Technology

- Elliott, R. Paul. 1965. Bacterial growth on food spoilage. Canner/Packer 134(3), pp. 30A-30B.
- Elliott, R. Paul, and Heiniger, Patricia K. 1965. Improved temperature-gradient incubator and the maximal growth temperature and heat resistance of Salmonella. Appl. Microbiol. 13(1), pp. 73-76.

1/ Research supported by P.L. 480 funds.



- Elliott, R. Paul, and Michener, H. David. 1965. Factors affecting the growth of psychrophilic microorganisms in foods--a review. USDA-Tech. Bull. 1320, pp. 110.
- Elliott, R. Paul, Straka, Robert P., and Garibaldi, John A. 1964. Polyphosphate inhibition of growth of pseudomonads from poultry meat. Applied Microbiol. 12(6), pp. 517-522.
- Hanson, Helen L., Brushway, Marian J., and Lineweaver, Hans. 1964. Flavor studies of irradiation-sterilized chicken. Food Technol. 18(11), pp. 141-146.
- Hanson, H. L., and Klose, A. A. 1964. When do consumers consider turkey meat tough? Poultry Meat, Broiler and Turkey Ed., 1(1), pp. 106-107, 116.
- Klose, A. A. 1964. Frozen foods--recent advances in science and technology. Proc. Internatl. Cong. Refrig. (11th) Munich, Germany, Aug. 27-Sept. 4, 1963, pp. 49-54.
- Klose, A. A., Campbell, Agnes A., and Hanson, Helen L. 1964. Effect of polyphosphates in chilling water on quality of refrigerated and frozen turkey meat. Proc. Internatl. Cong. Refrig. (11th), Munich, Germany, Aug. 27-Sept. 4, 1963, pp. 789-794.
- Rockwell, W. C., Jaufman, V. F., Lowe, E., and Morgan, A. I., Jr. 1965. Hex-tube freeze-dryer permits continuous F-D. Food Engin. 37(4), pp. 49-51.
- White, Elizabeth D., Hanson, Helen, Klose, A. A., and Lineweaver, Hans. 1964. Evaluation of toughness differences in turkeys. J. Food Sci. 29(5), pp. 673-678.

EGGS - PROCESSING AND PRODUCTS  
Western Utilization Research and Development Division, ARS

Problem. The nearly \$2 billion egg industry is periodically faced with burdensome surpluses that drive prices below the break-even point for many producers. The industry is also faced with declining per capita consumption. The estimate for 1965 is down 20% from the 1947-1949 average consumption. Because the demand for table eggs is inelastic, the increased utilization of eggs must come in the form of new egg products that compete by means of quality and convenience. Adequate knowledge is lacking of the properties, processing characteristics, and new product potentials of eggs to develop new markets. Present outlets for the 10% of egg production that is frozen or dried include the baking, confectionery, salad dressing, noodle, and baby food trades. Modified and new products emphasizing quality and convenience are needed to increase acceptance of egg products by these industries and to compete successfully with egg substitutes.

Increased utilization of eggs would not only benefit the producer, but would also diminish our feed grain surpluses since poultry and egg production account for about one-fourth of all grain fed to animals. Improved egg-containing products would benefit the producer in three ways: by providing an increasingly useful buffer for stabilizing egg prices; by providing additional uses and outlets for eggs; and by providing more remunerative outlets for wholesome eggs that are unsuitable for table use because of appearance or handling characteristics.

Egg processors have four general problems. First, the potential of yolk-containing solids in convenience foods can be fulfilled only with improvement of flavor stability, of dispersibility, and freedom from pathogenic Salmonella bacteria. Secondly, the processing costs of whites should be reduced and their utility improved. Third, further basic research on egg composition and components is essential to reach an understanding of physical and chemical changes induced by processing and storage and thus provide a rational basis for devising improved processes and products. Fourth, formulation studies designed to incorporate eggs into new household and institutional convenience products, are needed. This last study must encompass a full appraisal of physical, chemical, and microbiological problems peculiar to the formulated products.

USDA AND COOPERATIVE PROGRAM

In the Western Utilization Research and Development Division, a broad program of basic and applied research is conducted at the Division headquarters at Albany, California; by contract in Ames, Iowa, Ithaca, New York, and Davis, California; and by grant funds under P.L. 480 in France, Australia, and India. Fundamental research is conducted on egg proteins and their relations to the functional properties and quality of eggs, on egg lipids



and their role in off-flavor development in yolk solids, on the mechanism of bacterial penetration and survival in eggs, and on the bactericidal, anti-septic, anti-inflammatory, and food preservative properties of lysozymes and other components from eggs. Applied research is conducted on the stabilization of yolk-containing solids to increase the usefulness of eggs in dry mixes and other convenience foods, on new and improved drying procedures to make dried egg fractions and products more readily and more completely dispersible, on various methods of controlling Salmonella in eggs, and on factors in the handling of shell eggs that affect egg product quality and cost.

The Federal program of research in this area totals 13.9 professional man-years, including contracts and grants equivalent to approximately 3.4 professional man-years per year. Of this number 3.8 are assigned to chemical composition and physical properties, and 10.1 to new and improved food products and processing technology. In addition, three research grants on basic problems are supported by P.L. 480 funds.

#### PROGRAM OF STATE EXPERIMENT STATIONS

State stations maintain a continuing long-term program of basic and applied research on egg and egg product utilization. Its scope extends from concern for the quality of the freshly laid egg to research designed to maintain the quality and enhance the storage life of novel egg products.

The program of work concerned with maintenance of initial egg quality includes evaluation of: the influence of breed and strain of laying hen; the effects of diet; and variations in management and seasonal factors upon the physical and chemical properties of eggs and the relationship of egg constituents of the functional properties of the eggs. For example, the effects of different feed additives on yolk color and the subsequent quality of sponge cakes are being studied. The sponge cakes produced from light-colored and dark colored egg yolks are being compared by objective measurements of moisture, specific gravity, index of volume, compressibility and elasticity. Other research is concerned with determining the influence that shell characteristics, temperature, humidity, holding time in storage, washing, shell treatment and packing have upon egg quality and use. The relationship of shell thickness and breaking strength to retention of internal egg quality continues to receive special attention. The occurrence of pesticide residues in eggs and their elimination from eggs and egg products receives continuing study.

The frequent presence of salmonellae in feed and the implication of eggs as a source of salmonellae food poisoning is of great concern to the industry. Work which seeks to determine the source(s) of contamination of egg products is in progress. Data indicate that most Salmonella species occur not as egg interior contaminants but as egg shell contaminants. It would seem necessary, therefore, to develop methods for eliminating salmonellae at the processing plant in addition to elimination from the feeds and farms in general. Significant work is being carried out on egg product pasteurization efficiency



as affected by pH and other factors. This work has previously shown that Staphylococcus and Salmonella organisms have maximum heat resistance near pH 5.0. Related research has shown that the pH at which liquid whole egg is pasteurized and centrifuged affects its physical, chemical and functional properties. In other studies, the fluorescent antibody technique is being used to attempt to identify small numbers of Salmonella organisms in eggs.

Basic studies designed to more fully characterize egg composition, structure, biological activity and functional properties continue. A comprehensive investigation of the comparative biochemistry of the proteins of eggs is going forward. Investigations of the cholesterol and other constituents of eggs and factors affecting these constituents are continuing to provide useful information related to public health aspects.

Microbiological studies continue to include concern for bacterial spoilage of shell eggs, mode of entry of organisms into eggs, action of proteolytic and lipolytic enzymes of psychrophiles in deterioration of eggs and development of control methods. Other work relates to controlling bacterial contamination in new products.

Processing research includes evaluation of cleaning, washing and pasteurizing procedures. The effect of spray-drying egg white at various pH levels and the subsequent influence upon the resulting product continues to receive intensive study. Fractionation of egg proteins and other components has revealed that a number of changes occur due to freezing and spray-drying.

Product development work continues to seek new and novel egg products. Shell-less eggs, frozen eggs, frozen egg products and egg combination products receive some attention.

The costs, efficiency and economic feasibility of egg processing operations continue to be researched. These engineering economic studies are essential to evaluation of alternative methods of processing and utilization of eggs.

A portion of the work on egg utilization is carried out under the regional project NCM-40.

There are about 10.5 professional man years devoted to egg utilization research at the State experiment stations.

## PROGRESS -- USDA AND COOPERATIVE PROGRAMS

### A. Chemical Composition and Physical Properties

1. Egg Proteins. To facilitate the study of the nature of changes caused by processing and other treatments, work has been conducted on the development of feasible chromatographic methods of separating egg white proteins. Diethylaminoethyl cellulose (DEAE cellulose) has given the best resolution of the egg white proteins. At least 18 components can be detected by the procedure developed, but the fractions are not analytically pure.



Nevertheless, the separation patterns are sufficiently reproducible to show difference between the composition of individual eggs.

In studies of the effect of pasteurizing temperatures on egg white proteins it was observed that lysozyme is much less stable in egg white than it is when separated from egg white. Basic study of this phenomenon showed that it is the ovalbumin component of egg white that causes lysozyme to be less stable to heat in egg white than in buffer. The inactivation reaction occurs more rapidly at alkaline pH values and is quite rapid under conditions that do not denature either ovalbumin or lysozyme. Comparative studies with cysteine (an -SH compound) strongly indicate that the -SH groups of ovalbumin rapidly reduce the S-S bonds of lysozyme at the pH of egg white when the temperature is raised to 60° C or above. The possibility that the newly discovered reactivity of the -SH groups of ovalbumin influences the stability of egg white under milder temperature conditions is being investigated.

A study of the physical chemical properties of the unique egg white component, ovomucin has been initiated. Ovomucin is a complex mixture of mucoproteins and is reputed to play a major role in establishing the viscosity and the thick-white properties of egg white. With physical chemical methods now available it should be possible to define the basic properties of ovomucin and to relate these to the gross properties of egg white and egg products.

Basic investigations of lysozymes from eggs and other animal sources are continuing at the University of Paris, France, supported by P.L. 480 grant. Lysozymes are proteins with enzymic, bactericidal, and other biological properties. To throw light on the structure of the active site in the lysozyme molecule, purified lysozymes from hen eggs, human blood leucocytes, goose eggs, and turkey eggs are being compared as to substrate specificity of the enzymic activity. Enzymic action in the digestion of mycobacteria cell walls indicates that the most basic lysozyme from eggs reacts more rapidly than does the enzyme of human origin, which is slightly less alkaline.

Research supported by P.L. 480 funds at the Indian Institute of Science in Bangalore is concerned with changes in properties of egg yolk proteins resulting from freezing. The gelation of egg yolk after freezing and thawing is specifically under investigation. Protein-splitting enzymes such as chymotrypsin, trypsin, and papain, as well as collagenase and a crude phospholipase isolated from Russel's viper venom inhibited gelation, whereas carboxy peptidase had no effect. Another phospholipase from a bacterial source did not inhibit gelation although it increased the opacity of the yolk plasma. Basic peptides were released from egg yolk proteins by the proteolytic enzyme treatment. Amino acid analysis indicated that the released peptides contained lysine and arginine, probably released as terminal groups from the protein molecules.

Studies of the mechanism of transformation of ovalbumin into S-ovalbumin were initiated, with P.L. 480 funds, at the Commonwealth Scientific and Industrial Research Organization, Ryde, New South Wales, Australia.



S-ovalbumin is more heat-stable than ovalbumin from which it is formed. To elucidate their structural differences, two approaches are being used: one involves the use of a denaturing solvent, formamide, which causes the organized protein molecule to unfold or stretch out into a random-coil chain. If the difference between ovalbumin and S-ovalbumin involves only internal structure and a difference in the weak bonds that hold the chain in the folded state, the two proteins should be identical when completely unfolded. Ovalbumin and S-ovalbumin, however, are different when completely denatured with formamide. The difference is reduced by adding a reagent that breaks the chemical bond between sulfur atoms of two amino acids. Therefore, the difference appears to be related to the position of the disulfide cross-linking. The second approach is designed to test the hypothesis that the S-S bonds of the two proteins are in different positions. If this is the case, enzymic hydrolysis of the proteins should yield polypeptides of differing composition because the amino acids adjacent to the different S-S bonds would almost certainly be different. Only those fragments that might be involved in a disulfide cross-link are being examined.

## B. New and Improved Products and Processing Technology

1. Bacterial Spoilage of Eggs and Egg Products. An improved method has been developed for pasteurizing egg white to provide positive control of Salmonella, even under high levels of contamination. Pasteurization temperature for untreated egg white is limited by the instability of the protein conalbumin, which coagulates below the kill temperature for Salmonella. Iron-conalbumin is known, from our fundamental protein studies, to be more heat-stable than is conalbumin. The iron in egg yolk contributes to conalbumin stability so that whole eggs can be pasteurized without undesirable protein coagulation. We found that aluminum salts also stabilize conalbumin against heating, and they are more desirable than iron is for treating food, because they do not cause a color change in the product. Thus, pasteurization temperatures for egg white can be increased to provide Salmonella kills of inoculations as high as  $10^8$  with only minor damage to the functional properties of the egg white. Pasteurization at  $60^\circ$  to  $62^\circ$  C. is possible without coagulation of protein and without loss of volume in cakes made from the pasteurized eggs. However, increased whipping time is required. Where the whipping time increase is undesirable it can be overcome by adding a whipping aid, such as triethyl citrate. Commercial-scale pasteurization trials, using the aluminum addition, were made in several large processing plants. Over 30 concerns have applied for licenses to operate under the patent application. Pasteurized, Salmonella-free egg white is available from a number of processors. Eventually wide commercial adoption of this pasteurization treatment seems inevitable because of the simplicity of the treatment and the rapidly growing concern for Salmonella poisoning in foods.

Contract research to determine factors contributing to Salmonella contamination is continuing at Iowa State University in Ames. Feeding viable Salmonella to laying hens did not produce contaminated eggs. In processing plants, studies of work interruption of short or long duration for equipment



cleanup indicated that total counts in broken-out eggs may build up if clean-  
is superficial. The use of chemicals to pasteurize eggs is being investigated  
under contract research at the University of California at Davis. Purified  
iron conalbumin, high-quality ovomucoid, and antibodies in the form of rabbit  
antisera to whole chicken egg and to crystalline conalbumin will be used to  
determine the effects of chemical pasteurizing agents on the chemical, enzyma-  
tic, and biochemical properties of egg white constituents. A research grant  
to Cornell University was made for studies on the selenium metabolism of  
Salmonella because these microorganisms grow more readily on selenite media.  
Relative growth of Salmonella and E. coli on low-sulfur media indicate that  
selenium stimulates Salmonella growth but inhibits E. coli. The selenium-to-  
sulfur ratio may be critical in the development of selective media for  
Salmonella isolation and analysis.

2. Egg Powders. Functionality and stability of egg powders as affected by  
the addition of carbohydrate and by several processing variables have been  
studied. Gas-injection spray-drying, tested on a commercial scale at two  
processing plants, improved flowability and dispersibility of the powders.  
Bulk densities were lower and particle size was larger than for spray-dried  
egg powder without gas injection. Whole egg and yolk solids of various  
formulations were also prepared by forced-air drying of mechanically pre-  
formed foams and agglomeration of conventional spray-dried powders. Addition  
of carbohydrate and acid to egg white stabilized the dehydrated product and  
avoided the usual deglucosing step. The new product is superior to untreated  
spray-dried white in flavor and functional properties and is comparable to  
spray-dried deglucosed egg white. The increase in adventitious bacterial  
contamination that takes place in the usual deglucosing process can be avoided  
because the egg white can be dried immediately after carbohydrate and acid are  
added.

3. Precooked Frozen Foods. The principles governing the reactions of  
ingredients of prepared foods subjected to frozen storage were investigated.  
An increase in the use of eggs in such products is being sought through  
studies of the stability on freezing of foods involving egg foams. Both  
whole-egg and egg-white dessert soufflés have been studied. Egg-yolk con-  
centrations ranging from 9 to 13% and salt concentrations from 0 to 0.6% did  
not affect the volume of whole-egg soufflés. Sugar helps stabilize egg-white  
foam. Sugar concentration and its distribution between yolk and white  
portions of foams affect both the original volume and the storage stability.  
For products low in sugar, we have found commercial stabilizers that improve  
the frozen storage stability of soufflés. Methyl cellulose was superior to  
other commercial stabilizers tested.

## PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Chemical Composition and Physical Properties

Jauregui-Adell, Juan, and Jollés, Pierre. 1964. Contribution a l'etude du centre actif du lysozyme de blanc d'oeuf de poule: Action de l'acide iodacétique a pH 5.5 (Contribution to the study of the active center of lysozyme of chicken egg white: action of iodoacetic acid at pH 5.5). Bulletin de la Société de Chimie biologique, Extrait du Toma XLVI, No. 1, pp. 141-147. 1/

Jollés, Pierre, Jauregui-Adell, Juan, and Jollés, Jacqueline. 1964. Le lysozyme de blanc d'oeuf de poule: Disposition des ponts disulfures (Lysozyme of chicken egg white: disposition of the disulfide bonds). C. R. Acad. Sc. Paris, t. 258, pp. 3926-3928. 1/

Privett, O. S., Romanus, Olavo, and Kline, Leo. 1964. Ultraviolet absorbancy of volatiles as a measure of oxidative flavor deterioration in egg powders. Food Technol. 18(9), pp. 239-242.

New and Improved Products and Processing Technology

Carlson, C. W., Halvorson, A. W., and Kohler, G. O. 1964. Some effects of dietary pigmenters on egg yolks and mayonnaise. Poultry Sci. 43(3), pp. 654-662.

Haglund, John R., Ayres, John C., Paton, Alan M., Kraft, Allen A., and Quinn, Loyd Y. 1964. Detection of Salmonella in eggs and egg products with fluorescent antibody. Appl. Microbiol. 12(5), pp. 447-450. (Contract)

Hanson, Helen L. 1964. Recent research on prepared frozen foods. J. Amer. Dietet. Assoc. 46(6), pp. 523-525.

Hanson, H. L., and Fletcher, L. R. 1965. Preparation of pre-cooked frozen poultry products. U.S. Patent No. 3,169,069.

Ijichi, Kosuke, Hammerle, Olivia, A., Lineweaver, Hans, and Kline, Leo. 1964. Effects of ultraviolet irradiation of egg liquids on Salmonella destruction and performance quality with emphasis on egg white. Food Technol. 18(10), pp. 124-128.

Kaufman, V. F., Rockwell, W. C., and Lowe, E. 1964. Finned tube dryer speeds tumbling freeze-drying. Food Engin. 36(7), pp. 58-61.

1/ Research supported by P.L. 480 funds.



- Kline, Leo, and Sugihara, T. F. 1964. Drying of yolk-containing egg liquid. U.S. Patent No. 3,162,540.
- Kline, Leo, Sugihara, T. F., and Meehan, J. J. 1964. Properties of yolk-containing solids with added carbohydrates. J. Food Sci. 29(6), pp. 693-709.
- Kline, Leo, Sugihara, T. F., and Meehan, J. J. 1965. Preparation of dried egg white. U.S. Patent No. 3,170,804.
- Launer, Herbert F., and Hammerle, Olivia O. 1965. UV-dosage measurements in a geometrically complex system with light-sensitive paper. Quantum yield for Salmonella kill in egg white. Photochem. and Photobiol. 4(2), pp. 265-267.
- Lineweaver, Hans. 1965. Why it's now feasible to pasteurize egg whites. Proc., 1965 Inst. Amer. Poultry Indus. Fact Finding Conf., Kansas City, Mo.
- Sugihara, T. F., and Kline, L. 1964. Making cake doughnut mixes with yolk-containing egg products. U.S. Patent No. 3,152,910.
- Tomimatsu, Yoshio. 1965. A light-scattering study of the aggregation of ovalbumin near its isoelectric pH. Biochim. Biophys. Acta 94(2), pp. 525-534.
- U.S. Department of Agriculture. 1965. WRRL process for pasteurizing liquid egg white. Agr. Res. Serv. CA-74-14.

### III. MARKETING AND ECONOMIC RESEARCH

#### POULTRY PRODUCTS - MARKET QUALITY Market Quality Research Division, ARS

Problem. Technological developments continue in the poultry industries and create many new problems relating to the market quality of poultry and egg products. Introduction of highly mechanized equipment and machinery plus new techniques in processing affect the absorption and retention of moisture of ready-to-cook poultry, the contamination of poultry and egg products by spoilage microorganisms, the physical damage to poultry carcasses, and the sanitary and functional properties of egg products. To maintain quality of these products in marketing channels, more information is needed regarding the effects of the new technology as well as changes that occur during transportation and storage. In addition, objective methods of quality evaluation are needed for use in developing improved criteria and standards for inspection and grading to insure uniform, standardized, and wholesome products.

#### USDA PROGRAM

The Department has a continuing program involving food technologists and bacteriologists engaged in basic and applied research in the quality evaluation and quality maintenance of poultry products. The research is conducted at Beltsville, Maryland, and through a research contract with the University of Iowa, Ames, Iowa.

The Federal scientific effort devoted to research in this area totals 6.0 professional man-years, about equally divided between objective measurement and evaluation of quality, and handling, packaging, and storage.

Project MQ 3-22(c) "Effect of various disease syndromes on wholesomeness of market poultry" was terminated during this period.

#### PROGRAM OF STATE AGRICULTURAL EXPERIMENT STATIONS

State stations conduct a continuing program of research directed to improving or maintaining market quality of poultry products. A new regional project, NCM-40, Improving Acceptability, Stability, and Utilization of Poultry Products, includes portions of the work aimed at determining basic causes of quality losses and at finding methods for minimizing these losses. Development of methods of measuring quality and quality changes as well as evaluation of consumer acceptability of products also receives attention in the regional study. The regional effort is about equally divided between problems on eggs and on poultry meats.



A large number of studies related to determining the influence of genetic, environmental, and general management factors on the ultimate market quality are in progress. These studies often involve the team approach with both production and product scientists cooperating.

The consumption of ready-to-eat poultry and poultry food products has increased dramatically during the past few years. As a result, many investigations are concerned with study of the factors perceived by consumers as important quality attributes in fresh, frozen, and further processed items. With broilers, these studies relate mainly to factors of tenderness, flavor, and shelf-life; with eggs, interest has centered on shell quality, interior quality and functional properties. Several other studies are concerned with protection of product quality from the standpoint of public health or safety and wholesomeness.

The research effort devoted to market quality research on poultry products is about 30.9 professional man years.

## REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

### A. Quality measurement and evaluation of quality

1. Effect of Various Disease Syndromes on Wholesomeness of Market Poultry. The relationship of post mortem lesions commonly accepted as criteria of septicemia to known stages of septicemia was studied. Correlation of selected lesions (degeneration of pericardial fat, degeneration of the anterior edge of the thigh, dark, and dehydrated muscle, markedly enlarged liver, markedly enlarged kidney) with septicemia was shown to vary with the lesion and the length of time the bird was in a septicemic state. A total of 17 trials (14 with chicken fryers, and 3 with turkeys) involving inoculation of birds at various ages with particular combinations of disease agents, and then determining the nature and severity of symptoms and lesions at the peak of infection and then at time of slaughter were completed. A final report was submitted and the project terminated. Several manuscripts have been accepted for publication.

(MQ 3-22(c))

2. Methods to Evaluate Shelf-Life of Eviscerated Poultry. Accuracy of Bacterial Sampling of Chickens. The effectiveness of the swab method of determining microbial populations on surfaces of ready-to-cook chickens was shown to be dependent on the chicken part that was sampled. Total aerobic microorganisms were more predominant on thighs than on breasts or drumsticks of fryer chickens. There was no significant difference in the bacterial counts of swab samples from thighs, drumsticks, and breast between the right and left side of fryer chickens. There was no significant difference in bacterial counts obtained from several birds within a lot as long as the same part of each bird was sampled. A manuscript was prepared and submitted for publication.

A comparison of 2 standard methods for preparing dilutions for agar plate counts (successive 1:100 dilutions and successive 1:10 dilutions) indicated that there was no significant difference in "average" bacterial counts between the two methods. These studies were carried out on "drip" from two groups of ready-to-cook chickens stored at 34°F.

(Exploratory Work)

3. Detection of Chlorinated Hydrocarbon Pesticide Residues in Poultry and Eggs. Simplified procedures for analysis of poultry and egg products for chlorinated insecticide residues using thin-layer chromatography (TLC) were studied. Whole egg or yolk, either liquid or dried, was blended with carbon celite using ethyl ether, and the mixture was added directly to the columns, simplifying sample preparation. Use of suction speeded column flow without affecting results. Cleanup of residues in rendered chicken fat was successful on either carbon-celite or partially inactivated florisil columns. With TLC it was possible to detect 0.02 micrograms or less of DDT, DDE, DDD, dieldrin, endrin, methoxychlor, lindane or heptachlor epoxide in 0.5 g egg (fat basis) or 0.4 g chicken fat. Time required for the complete analysis was less than two hours for either type of sample. A manuscript was prepared and has been submitted for publication.

(MQ 3-70)

B. Quality maintenance in handling, packaging, and storage

1. Chilling of Eviscerated Broilers. The relation of time and temperature of commercial continuous chilling of fryer chickens to the bacteriological condition and shelf-life of the carcass was determined. Chickens removed from various locations along commercial chillers were examined bacteriologically in the plant and throughout storage of up to 15 days at 34°F. Chickens chilled to about 40°F in a length of a specified continuous chiller were not of lower bacteriological condition or shorter shelf-life than chickens chilled to lower temperatures in greater lengths of the same type of chiller.

(Exploratory Work)

2. Quality Retention of Eviscerated Poultry as Related to Method of Slaughter. The radioactive tracer ( $I^{131}$ ) technique of mammalian blood volume determination was adapted for studies with chickens. Birds weighing 1,000 g contained about 11.6 percent of that weight as blood whereas birds weighing 2,000 g had blood volumes of about 7.3 percent of their body weight. Chickens slaughtered by six methods (standard cut, kosher cut, and standard cut following brain stick, CO<sub>2</sub> immobilization, physical stun, and electric shock) lost 35 to 45 percent of their total circulating blood during 90 sec. bleeding. Blood loss in both 90 and 300 sec. bleeding was greatest in the physically-stunned birds and the standard-cut birds.



Method of slaughter did not significantly affect the amounts of blood retained in breast, thighs, drumsticks, or wings, but did affect the amounts retained in various organs of the viscera. Manuscripts have been prepared and submitted for publication.

(MQ 2-81)

3. Radiation Pasteurization of Tray Packed Cut-Up Chickens. Statistical analysis of off-odor scores of stored irradiated (0.1 and 0.3 Mrad) chickens was completed. For chickens which had been irradiated in the fresh condition and subsequently stored at 34°F and 40°F, the analyses indicated that, on the average, over a 15-day storage period there were no significant differences in either raw and cooked off-odor scores between chickens irradiated at the two levels or stored at the two temperatures. This was also true for chickens which had been irradiated in the frozen condition and stored up to 12 days at the two temperatures. Chickens irradiated in the frozen state with 0.5 Mrad and subsequently stored either at -15°F or 40°F exhibited a greater degree of oxidative deterioration (raw off-odor scores TBA values) than either 0.1 Mrad or unirradiated control chickens. Storage of the 0.5 Mrad chickens for 27 days at 40°F produced higher TBA values than similarly treated birds stored at -15°F for periods up to 14 weeks. TBA values and off-odor scores of chicken legs from 0.1 Mrad and 0.5 Mrad irradiated frozen carcasses cooked at 180°F for 50 minutes and then stored at 40°F for 16 days were not significantly different from unirradiated controls.

(Exploratory Work)

#### PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

##### Quality Measurement and Evaluation of Quality

Mercuri, A. J., and A. W. Kotula. 1964. Relation of "Breast Swab" to "Drip" Bacterial Counts in Tray-Packed Chicken Fryers. J. of Food Science 29:854-858.

(Exploratory Work)

Sadler, W. W. and R. E. Corstvet. 1965. A Second Survey of Market Poultry for Salmonella Infection. Applied Microbiology 13(3):348-351.

(MQ 3-22(c))

##### Quality Maintenance in Handling and Packaging

Rowan, J. D. and A. W. Kotula. 1964. Portable Power Supply Simplifies Air Testing. Food Engineering 36:73.

(MQ 2-10)

Kotula, A. W., J. A. Kinner, and J. D. Rowan. 1964. Portable Sampler for Airborne Microorganisms. USDA Agric. Marketing, Oct. p.4.

(MQ 2-10)

Thomson, J. E. 1964. Effect of Polyphosphates on Oxidative Deterioration of Commercially Cooked Fryer Chickens. Food Technology 18(11):147-148.

(MQ 2-10)



POULTRY AND EGGS - MARKETING FACILITIES,  
EQUIPMENT AND METHODS  
Transportation and Facilities Research Division, ARS

Problem. A continuing increase in the production of poultry, the inauguration of the Poultry Products Inspection Act, and the rapidly developing demand for further processed poultry items in the United States has resulted in significant changes in marketing methods and requirements for poultry of all classes. Similarly the rapid expansion in the number of large-scale commercial egg farms marketing large lots of uniform fine quality eggs has called for drastic changes in the marketing methods and requirements for eggs and egg products. These changes have had an impact on poultry processing and egg grading and packing plant operations throughout the country, especially as these are related to facilities, equipment, and work methods. Problems in this connection generally have been worked out by the industry on an individual plant basis when solutions have been attempted. In the main, the answers have furnished only a temporary relief in the area of expansion needs and have been of little help in developing guidelines for new plants or solving general plant production problems and problems involving new processes. Although the Department research in this area has been successful in developing some of the basic guidelines that industry needed and that are now being effectively used by many plant operators, continued improvements in operating efficiency in existing facilities, effective assistance in planning new or expanded facilities needed in many of the recent food processing trends; and the development of improved plant layouts, devices, and equipment, and work methods for the handling, processing, grading, and packing of poultry, eggs and egg products in this changing environment; will necessitate the continuation and expansion of this research effort.

USDA PROGRAM

The Department has a continuing long-term program involving engineers and marketing research analysts engaged in both basic and applied research to develop more efficient work methods, techniques, operating procedures, devices, and equipment and to design improved facilities for the handling and preparation of poultry, eggs, and egg products for market. Research on chicken-class poultry processing operations is carried on in commercial poultry processing plants in the Southeast by Department personnel at Athens, Ga., in cooperation with the Georgia Agricultural Experiment Stations. Contract research to determine the optimum production rates in chicken processing plants for the eviscerating and federal post-mortem inspection operations and is being conducted by the American Scientific Corporation at Alexandria, Va., in selected poultry processing plants on the Delmarva Peninsula. Research on turkey-class poultry processing,

egg products processing, and cleaning, grading, and packing shell eggs is carried on in West Coast turkey processing and in egg grading and packing plants, in cooperation with the California Agricultural Experiment Station at Davis, Calif.

The Federal effort devoted to research and development work in this area during the fiscal year 1965 totaled 7.0 professional man-years; 4.9 man-years (including 1.9 man-year of contract work and 0.1 man-year under a research cooperative agreement with the Georgia Agricultural Experiment Stations) on poultry; 1.1 man-years (including 0.1 man-year under a research cooperative agreement with the Regents of the University of California) on shell eggs and egg products; and 1.0 man-year on program leadership.

## REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

### A. Methods, Equipment, and Facilities for Grading and Packing Eggs

This research concluded in Hyattsville, Md., last year was directed toward reducing egg marketing costs through engineering and marketing research involving a systems analysis of existing work methods and equipment for grading and packing eggs, and the development of improvements. A report of the results of this study "Evaluation of Mechanized Egg Grading and Packing Equipment" has been reviewed and is being edited for publication. It will provide guidelines for egg grading plant operators in determining the degree of mechanization they desire in their operation and in selecting combinations of mechanized egg grading and packing equipment that will yield the greatest output per worker and equipment dollar at specified production and product quality levels.

### B. Improved Methods, Techniques and Equipment for Cleaning Eggs

This research conducted at Davis, Calif., involved the development of techniques and equipment that will do an effective in-line job of cleaning shell eggs with minimum quality deterioration and breakage, and that will minimize labor requirements. The basic studies of typical spoilage organisms to determine time and temperature conditions necessary for safe, commercial egg washing, and the testing of commercial washers; together with the design, construction, and testing of an experimental washer reported last year have been completed and are now in manuscript form. This report on "The Bacteriological, Chemical and Physical Requirements for Commercial Egg Cleaning" has been reviewed, edited and prepared for publication. The report shows that by applying the time and temperature parameters determined in the laboratory and by developing a cleaner that uses some of and either improves or adds to the cleaning mechanics of commercial machines; almost 70 percent more eggs can be cleaned with less hazard to egg spoilage than is now being done in commercial operations.



Field tests of the experimental cleaner, conducted in a commercial egg grading and packing plant under regular operating conditions largely confirmed the laboratory findings. The cleaning effectiveness of the experimental equipment, under commercial conditions, averaged 78 percent; compared with an average of 35.9 percent for commercial units. Shell damage due to cleaning action was reduced by almost 50 percent (to 1.89 percent checks) and spoilage, after holding for an extended period was essentially eliminated (0.6 percent). The results of the field tests have been prepared in manuscript form and are now being reviewed prior to editing and publication.

#### C. Improved Layouts and Engineering Designs for Egg Grading and Packing Plants

At Davis, Calif., this long-term research was directed toward the development of improved building designs and layouts for small egg grading and packing plants; including the preparation of engineering design drawings, the development of building specifications, and facility and equipment layouts. The recent trend in the poultry industry has been toward smaller egg grading and packing plants where the production of only a few large commercial flocks are handled. With this trend there has arisen a need for information on plant facilities including equipment arrangements, storage space requirements, egg cooling facilities and structural design guidelines for plant operators. The normal developmental pattern with these small plants, generally has involved a very small facility to begin with and then as flock production and size increased, the grading and packing plant capacity has been expanded. This created a need for layouts which are planned for possible expansion.

To meet these requirements; studies have been made of the overall layouts, equipment arrangements and other requisites, the space allotted for each particular area, and the site plans of a number of small commercial processing plants. From these studies, the basic requirements for dry material (cases and cartons) storage, egg storage for incoming receipts, storage space for graded eggs, the grading and packing area, and the building site have been developed. These data have been used to prepare preliminary layouts suitable for use with any of the more popular egg grading and packing equipment suitable for the volume levels included. Final layouts will be developed next to permit expansion from a range of 200 to 400 cases per day to 800 cases per day. In addition, the building specifications for a 400 case per day plant will be developed.

#### D. Development of Equipment and Methods for Pasteurizing Liquid Whole Eggs in Small Volume Breaking Rooms

This research which is part of the long-range program being carried on at Davis, Calif., is designed to meet the needs of a rapidly increasing number of small egg grading and packing plants, handling the production of several large commercial flocks, that have found it advantageous to break out, can,

and freeze undergrade eggs instead of marketing them in the shell. Although the Department has developed guidelines for facilities, equipment and layouts for small egg breaking rooms, with the recent emphasis being placed on pasteurization requirements for liquid eggs by both State and Federal authorities, an urgent need has developed for pasteurizing equipment that can handle the production from small volume operations as effectively as pasteurizing equipment presently employed in large volume plants that specialize in the preparation of egg products. The cost and large through put requirements of this equipment make it unsatisfactory for the small volume operations.

This research was initiated at the end of the report year to determine the feasibility of using batch-type equipment for small volume operations. It is being conducted under a research cooperative agreement with the University of California by its Department of Food Science and Technology. Development work will involve determining the feasibility of this type equipment from the standpoint of its effects of the pasteurization process and on the properties of liquid egg. Processing and production problems involved in pasteurizing small batches of liquid egg at intermittent intervals (instead of continuously) and possible costs of this process under commercial conditions also will be studied.

#### E. Improved Methods and Equipment for Cutting Up and Packing Chickens

At Athens, Ga., this research involves the study of the methods, equipment, and facilities for cutting up whole ready-to-cook chicken. The development of mechanized weighing and packing of broiler parts to exact weights reported last year was prepared as a marketing research report and was ready for publication as the year ended. Observations of the cut-up operation in a number of large chicken processing plants during the developmental work on mechanized packing indicates that: (1) There is a great diversity of methods, equipment, package types and end products throughout the industry because of differences between individual customer's product and package requirements; and (2) because of this diversity in the styles of packs, standard or basic chicken dismembering procedures have not been developed. In order to develop meaningful guidelines that can be applied industry-wide, research was undertaken on the methods of performing chicken carcass dismembering operations when the parts are cut to meet U.S. Standards of identity. A search of the literature was completed, U.S.D.A. specialists were consulted for interpretation of the regulations governing the standards of identity for chicken parts, and basic cuts were performed in the laboratory to develop an understanding of anatomical structure of the chicken carcass and to acquire the "feel" of proper cutting techniques. Case study plants were selected, methods analysis techniques were designed, and the gathering of labor requirements data was started.



#### F. Improved Designs for Chicken Processing Plants

This research at Athens, Ga., involving the development of improved designs and layouts for chicken processing plants has been completed. A manuscript now is in the process of preliminary review and clearance with the cooperators. When published the report will furnish guidelines essential to plant site selection and building design. Requirements for efficient layouts and services were developed for each of the component work and service areas within the plant. Practical application of the guidelines are illustrated in plant layouts that provide for expansion needs for handling substantial increases in production and permit diversion into further processing operations with minimum downtime and building expansion costs.

#### G. Improved Methods, Equipment and Facilities for Improving Chicken Processing Plant Efficiency Through Balance of Inspection and Eviscerating Operations

Under a contract with the American Scientific Corporation, Alexandria, Va., field studies were conducted in selected chicken processing plants of specified volume levels, equipment arrangements, and inspector combinations to evaluate both the processing and inspection labor and equipment requirements that yield the most efficient worker-inspector mix for plants processing 1,000 to 3,000 birds/hr., 3,000 to 5,500 birds/hr. and 5,500 to 8,000 birds/hr. Optimum line speeds, i.e., levels at which maximum production per worker and utilization of inspector(s), time is possible, were predicted from the data derived from time and motion studies. These predictions were then tested under operating conditions. The results have been prepared in final report form by the contractor for review and acceptance by the project supervisors. When accepted, the findings will be published as a guide to the Department Inspection staff and to poultry processing plant operators, in determining production levels, equipment types and inspector-processing crew mixes at which it is most economic to operate.

#### H. Improved Methods and Equipment for Handling Live Chickens by Commercial Processing Plants

At Athens, Ga., research involving studies of live chicken handling operations were conducted to determine the methods and equipment that cause the relatively high rate of bruising of live chickens prior to slaughter and to develop improvements that will reduce this costly damage to product quality. Downgrading of chicken carcasses because of bruises sustained during live handling were found to be substantial, ranging as high as 35 percent in some flocks and averaging between 15 and 20 percent of all birds processed. It was determined that there is a monetary loss of one dollar per 1,000 birds processed for each one percent in downgrades (not including meat loss). Accordingly a plant processing 60,000 birds per day a 20 percent downgrade would be sustaining a daily loss of \$1200. Preliminary figures indicate that 60 to 70 percent of the bruising occurs during the driving, catching, and cooping operations; 25 percent of which could be avoided by removing

obstructions such as feed troughs, water fountains, etc. before driving. Limited trials with a new style plastic coop resulted in a 2 to 3 percent decrease in bruising rates as compared with conventional wood coops. Other possibilities for reducing bruising such as improvements in driving and catching methods and truck loading and unloading procedures are planned. In descending order of importance the following are factors that contribute significantly to bruising rates: (1) Driving chickens within the producer house; (2) catching and cooping; (3) hauling to the plant; (4) unloading at the plant; and (5) hanging the live birds on the slaughter and defeathering line. This research also involves a determination of chicken reaction to external stimuli that might influence catching, handling, and cooping operations. This phase of the work is carried out by the Poultry Department of the University of Georgia under a research cooperative agreement. By use of telemetry equipment, pulse and respiration rates and blood pressure changes will be recorded to determine the feasibility of introducing stimuli that excite, calm, or attract the birds during the various handling operations. As the report year ended the first test flocks were being observed for reaction to extremely bright lights and extended periods of dim lighting. Telemetry equipment is being tested before use on individual birds in the test flocks. As significant results are documented an interim report will be prepared.

#### I. Improved Methods and Facilities for Chilling, Weighing, and Packing Turkeys

This research at Davis, Calif., covers the development of improved methods, equipment and facilities for chilling, weighing, and packing turkeys.

In most of the turkey processing operations, advances that have taken place in the broiler industry have been adapted to turkey processing. However, due to differences in carcass weight and consequently in handling labor and chill time the turkey processing operation encounters a bottleneck after evisceration. Further, major equipment companies have not actively engaged in equipment development programs for the packing area. This research is directed towards reducing the relatively high labor requirements for packing whole eviscerated turkeys and synchronizing these operations with the preparatory steps that precede them. Weighing and bagging stations designed and shop tested last year were tested in short runs under commercial conditions during the past turkey processing season. Based on the findings of these tests, the equipment was redesigned for extensive testing during the coming turkey season. Preliminary results indicate a potential for reducing the physical effort for the job involved and for reducing the crew size at the turkey packing stations by two or more workers on 1,200 bird per hour processing lines. Time and motion studies were made of existing methods of weighing, bagging, and weight recording. The results will be compared with the results of the tests conducted with the experimental bagging equipment and then prepared in report form.



## J. Improved Methods, Equipment and Facilities for Preparing Turkey Specialty Items

This research at Davis, Calif., is directed toward the development of improved methods, equipment and facilities for preparing many of the specialty items currently being marketed from cut-up ready-to-cook whole turkeys. The recent sharp increase in demand for turkey specialty items prepared from deboned turkeys, has meant a tremendous growth in the production of these products. Due partly to the many variations of these items and the low return type investment for equipment manufacturers, (mainly cutlery) there has been a lag in the development of improved methods and techniques for performing the operations. This research is directed towards a systems engineering analysis of turkey specialty operations with initial emphasis being placed on: (1) The development of improved methods of presenting the carcass for deboning; (2) improved labor efficiency and meat yield in deboning; and (3) improved tools and devices for removing meat from the carcass. Work was begun on all three of these phases during this report year. A prototype rigid shackle to support the carcass in a firm manner has been constructed. Experimental testing of air powered knives has been undertaken and an evaluation of the present dismembering and deboning operation has been started. Time studies of existing deboning operations and methods analysis work required for the development of holding devices and powered tools are underway.

## PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE PROGRAMS

### Methods, Equipment, and Facilities for Grading and Packing Eggs

Hamann, J. A. 1965. Mechanization in the Egg Grading and Packing Industry. A technical paper presented at a meeting of the Central California Egg Dealers Association, Oakland, Calif. April 1965.

### Improved Methods, Techniques and Equipment for Cleaning Eggs

Walters, R. E. 1964. New Design Features for Commercial Egg Washers. A technical paper presented at the Winter meeting American Society of Agricultural Engineers, New Orleans, La. December 1964.

Hamann, J. A. 1965. Wash Eggs? That is Not the Question. A technical paper presented at the Egg Marketing Institute, Cornell University, Ithaca, N. Y. May 1965.

Improved Methods and Equipment for Cutting Up and Packing Chickens

Childs, R. E. 1965. Mechanized Weighing and Sizing of Chicken Parts. A technical paper presented at the Southeastern Poultry Processor's Workshop, Athens, Ga. April 1965.

Improved Designs for Chicken Processing Plants

Childs, R. E. 1965. Chicken Processing Systems. A technical paper presented at the Summer Meeting American Society of Agricultural Engineers, Athens, Ga. June 1965.

Improved Methods and Facilities for Chilling, Weighing and Packing Turkeys

Walters, R. E., Hamann, J. A. 1965. Turkey Processing Operations. A technical paper presented at the Summer Meeting American Society of Agricultural Engineers, Athens, Ga. June 1965.

Improved Methods, Equipment and Facilities for Preparing Turkey Specialty Items

Walters, R. E. 1965. Variations in Turkey Deboning Operations. A technical paper presented at the Poultry and Egg Further Processing Conference, University of California, Davis, Calif. June 1965.





